

STUDIES ON THE METABOLISM OF GUM ARABIC.

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ABSTRACT

This thesis has studied the metabolic properties of gum acacia (arabic) (GA) a water-soluble form of dietary fibre, in long term and short term experiments in the rat and in short term experiments in man. Morphological effects on the intestine and pathological consequences of long term ingestion have also been studied as has the suitability of the rat as a model for the study of fibre metabolism.

GA administered to rats for a period of 2 years in variable dosage, and in high dosage for 90 days resulted in no adverse, dose-related, pathological, biochemical or haematological effects. Caecal size increased with addition of the polysaccharide. After 2 years, a significant linear relationship between decreasing jejunal crypt cell production rate and increasing dose of GA was demonstrated. Small and large intestinal morphology remained unaltered. Male rats had significantly reduced growth rates on high dose GA in short and long term experiments. Renal and liver weights also fell as a result of nutritional inadequacy without evidence of organ dysfunction.

GA was administered in a pellet and in an elemental diet which allowed the inclusion of dietary fibre. Faecal weight dropped markedly on elemental diet and rose little on addition of GA indicating extensive degradation. GA could be recovered from stomach and small intestine but not from caecum, colon or from faeces. GA administration resulted in significantly increased breath methane (CH_4) excretion in rats after 3 weeks on a pellet diet. Hydrogen (H_2) excretion remained unaltered. Caecal excision abolished this increase. Elemental diet abolished H_2 and CH_4 excretion but addition of GA restored H_2 and CH_4 excretion after an initial delay. Volatile fatty acid (VFA) excretion increased linearly with increasing GA ingestion. Caecal excision reduced VFA production. Acetate and butyrate proportions altered reciprocally with increasing dose of GA and with aboral passage of intestinal content. Elemental diet resulted in the appearance of isomeric VFA forms, usually absent.

In human studies GA resulted in a significant reduction in serum cholesterol after 21 days. No other significant effects on serum biochemistry, faecal characteristics or constituents occurred. Glucose tolerance was unaffected. Breath hydrogen excretion increased but methane excretion remained unaltered. GA could not be recovered from stool after ingestion.

GA has similar properties to other water soluble dietary polysaccharides though different responses occur in rat and man. Elemental diet results in some alteration in metabolic environment which may affect its use as a vehicle for fibre

study. Caution must be employed when the results of metabolic studies of dietary fibre in the rat are extrapolated to results in man.

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Studies of the metabolism of Gum Acacia (arabic) in the rat and man.

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Preface:

Dietary fibre (D.F.) is a complex mixture of substances which differ greatly in their chemical constituents, structure and physical properties. These are admixed in the normally consumed diet and therefore their effects on the host may be masked or altered by the presence of many other dietary interactions. Numerous studies have therefore been carried out in mixed dietary settings where supplements of dietary fibre have been added and their effects measured after relatively brief periods of time. With careful dietary monitoring and control useful data have been obtained though often with conflicting results. These discrepancies arise as a result of a number of factors which include: difficulties in standardising the physical and chemical properties of the D.F. administered, variations in the quantities of D.F. administered, the relatively short term, acute, nature of most dietary experiments, and the known wide variations in intestinal habit known to occur in individuals and between groups of healthy subjects. Furthermore in the past though chemically defined fibre isolates have been studied the majority of study has been directed at structurally and chemically complex, mixed fibre sources the major examples of which have been cereal and vegetable fibres. The effects of these fibres have been particularly well defined in their abilities to alter colonic and to a lesser extent small bowel dynamics though considerable evidence has been amassed as to their metabolic effects on the host. Isolates have in general been studied for their metabolic activities and effects on small bowel activity. Particularly in the assessment of metabolic activity there has been difficulty in defining the mechanisms of action particularly of the mixed fibre sources.

A number of authors have recognised these problems. The importance of adequate chemical definition of fibre sources to be studied has been emphasised. D.F. consists of a mixture of distinct chemical substances and should be defined and studied in these terms (Cummings 1981). If this is so then studies of isolates should be more frequent. Southgate (1977) suggested that to examine "dietary fibre" was inadequate and that the time had come to look at the performance of specific components included

in this definition. Having introduced the concept of dietary fibre, Trowel has arrived at an acceptable version of the almost impossible definition of D.F., (Trowel 1972, Trowel 1974, Trowel 1976, Trowel et al 1976) and has suggested that more attention should be paid to testing a variety of precisely defined dietary fibres. Such an approach has attractions, not least that chemical analysis remains the most definitive method of characterising fibre. Nevertheless the importance of physical properties and structure which are less easy to characterise emphasise the difficulties of the isolate approach and the complex interactions of effects that occur with D.F.

If then there is a need to study more closely the effects of chemically defined components of D.F. by what means should it be done? Clearly studies in humans are most applicable but are limited by the lack of access to the gastrointestinal tract other than by relatively indirect means of measurement of oral intake, faecal output, or by measurement of fermentation products such as hydrogen or methane. Of animal models used in fibre experiments the rat is most popular though doubts surround its suitability for comparison with effects in man. There is attraction therefore in studying the properties of a single, identifiable, D.F. polysaccharide in the rat and man and comparing its metabolic fate.

In order that detailed study may be made into the metabolic fate of a single complex polysaccharide component of D.F., the components of the diet in which it is included must be highly defined. Clearly in a mixed diet the admixture of many dietary polysaccharides and other complex molecules that pass substantially intact into the colon will make for great difficulties in distinguishing metabolic fates of individual polysaccharides. A completely absorbed (elemental) diet to which components of D.F. may be added will allow the delivery of the polysaccharide alone into the large intestine (along with a small quantity of unabsorbed substances of endogenous origin - mucus glycoprotein, desquamated epithelial cells) and thus permit its metabolic fate to be studied in isolation. Such an elemental diet would naturally need to provide the subject with an

adequate intake of nutrition despite the addition of graded quantities of D.F. polysaccharide.

Whilst the attractions of a diet allowing the study of the colonic metabolism of D.F. in isolation are apparent, there may be limitations to this method. The absence of other dietary components normally reaching the caecum may in altering the biochemical or bacteriological environment of the large intestinal lumen affect the metabolic fate of the isolate. Marked reductions in the entry of substrate into the large bowel lumen may alter bacterial populations and thus their metabolic effects on the D.F. isolate being studied. Elemental diets while allowing the study of single isolates may, in so doing, result in an alteration in the pathways of molecular degradation seen when the caecal and colonic lumen contains a heterogeneous mixture of biochemically active D.F. polysaccharides and other complex dietary substances present when a mixed diet is consumed. It will therefore be of some importance to determine whether such differences in metabolism exist, and if they do whether they are such as to diminish the value of elemental diets in the study of D.F. isolates.

Winitz et al (1970) have emphasised the importance of the use of highly defined diets when assessing the effects of substances on metabolism because of the complexity and ill-defined nature of most mixed diets. In a similar vein Southgate (1977) has suggested that if detailed progress is to be made D.F. may require to be examined as its individual chemical components. Nevertheless the effects of such elemental diets have been studied little with reference to the metabolism of complex polysaccharides.

A further problem in D.F. research is the length of time over which observations are made of its effects on the host. The great majority of research has been centred on relatively short-term experiments lasting for between one and three months. In these it is apparent that many effects may be attributed to D.F. and of these some (mineral balance for example) may appear to be potentially injurious to the subjects in the longer term.

Until now evidence for the beneficial and much less so for the deleterious actions of fibre have arisen from historical reviews of dietary habits and incidence of disease, or from epidemiological studies of populations with dietary habits which differ widely. From such comparisons has arisen the concept of the "dietary fibre hypothesis" (Trowel 1976) and of the role of D.F. in the aetiology of the "pressure" or "metabolic" diseases of Western Civilisation (Burkitt et al 1974, Burkitt 1975, Burkitt 1973, Cleave 1974, Cleave 1956). However such epidemiological evidence though valuable in pointing to potentially beneficial effects of D.F. in the long term is, because of the interaction of many other factors in the aetiology of these diseases, unreliable in pointing to a precise role for fibre. Long term prospective studies are therefore required. However these are impractical in man and the only approach is through long term animal studies of which there have been very few (Hegstedt 1977).

Substances which are used commonly in the preparation of foods are under increasing scrutiny from controlling bodies with regard to long term safety and effects of ingestion of these substances. Gum arabic is one such substance for which insufficient data are available to determine whether increased consumption would represent a dietary benefit or hazard (U.S. F.D.A. 1974), and for which there has recently been pressure to obtain such information in animals and man.

This study, therefore, was conducted with the following aims:

1. To study the metabolic fate of a dietary polysaccharide gum arabic that has been chemically defined and is readily identifiable using simple techniques.
2. To compare the metabolism of gum arabic in the rat and man and from this to judge the suitability of the rat model for the study of fibre metabolism.
3. To establish a method whereby rats could be maintained and thrive on a highly defined elemental diet into which gum arabic or other dietary fibres might be satisfactorily incorporated.

4. To determine the effects on large bowel metabolism of such an elemental diet and the effect of re-introduction of complex "unavailable" dietary polysaccharide using gum arabic as a model.
5. To examine the properties of gum arabic in the light of established knowledge of the effects of other components of dietary fibre in animals and man.
6. To assess in long term experiments in the rat the consequences of ingestion of gum arabic in varying doses and in shorter term sub acute studies to assess its safety in relation to established regulatory standards for ingestible substances.
7. In the following introduction the known properties of gum arabic will be reviewed and what little scientific study of its metabolic properties will be examined. In addition a review has been made of the knowledge of the behaviour of other components of D.F. where these are pertinent to the study of gum arabic. The place of gum arabic in the classification of D.F. is also discussed.

Introduction

Gum acacia (arabic) is a high molecular weight polysaccharide produced in the form of an exudate by the trees of the genus *Acacia* (leguminosae). The genus *Acacia* contains approximately 900 species and is found throughout the world. Gum acacia production is centred on Africa where approximately 200 species have been identified. Of these only two species provide gum in commercially significant quantities - *Acacia senegal* Willd. (syn. *verek*.) and *Acacia seyal* Del. Gum arabic as used commercially is a highly defined entity and in effect only *Acacia senegal* is now acceptable for use.

Acacia grows widely in arid and semidesert regions. The principal sites of production are, in order of volume of production, Sudan, Nigeria, Mali and Mauritius. Harvesting is by collection of naturally occurring gum arabic or by tapping of trees since it is an exudate produced in response to injury. Annual production is approximately 40,000 tons per year of which approximately 20,000 tons are used in confectionary manufacture. Annual production is roughly distributed in three equal shares between North America, Europe, and the Rest of the World.

Its properties as a binding agent, thickener, and emulsifier are widely used in food production and it finds widespread use in the confectionary industry. Average daily intake is difficult to estimate but clearly varies widely between subjects, and from day to day. In foodstuffs intake can only be regarded in small almost trace quantities but in confectionary intake may be substantially increased. Daily consumption may therefore vary from none to more than 20g.d^{-1} particularly in children and adolescents.

Though over 900 species of *Acacia* are known, the exudates of only approximately 100 species have been characterised (Anderson 1978). Before purification gum arabic presents as yellowish white to pale amber tear shaped nodules which have a glassy, transparent appearance. These nodules contain a significant quantity of

impurities such as sand, bark, and proteinaceous substances though these represent only 5% of its weight at a maximum. After commercial purification (described in Chapter II) gum arabic appears as a pure white powder.

The physiochemical properties of gum arabic have been extensively investigated (Anderson and Stoddart 1966). Gum arabic certainly is one of the most complex, naturally occurring, botanical polysaccharides known. It is a complex heteropolymer of four carbohydrate monomers - galactose, arabinose, rhamnose and glucuronic acid. Each acacia exudate differs in its ultra-structural appearance and the proportion of sugars present in the molecule. Thus the range of proportions of sugars is as follows: galactose 20-80%, arabinose 5-60%, rhamnose 0.4-14%, glucuronic acid 4-44%. Gum arabic has an average composition - galactose 36%, arabinose 36%, rhamnose 14%, glucuronic acid 14%.

The gum arabic molecule takes the form of a globular, spherical and highly branched structure. Its molecular weight is variable between 0.5×10^6 and 1.8×10^6 . Its central core consists of galactose with side chains of arabinose units. Terminal units are composed of rhamnose or glucuronic acid molecules. In solution approximately 50% of the carboxyl groups of the acid moiety are ionised and thus the external surface of the molecule presents a highly charged appearance to which cations may bind, principally calcium magnesium, sodium and potassium.

The molecule is highly soluble in water and practically insoluble in ethanol. A maximal aqueous concentration of 42% can be achieved. The resulting solution is acidic and a 10% solution laevorotatory.

The gum arabic solution is colourless. Gum arabic may be precipitated out of aqueous solution by addition of 95% acidified ethanol. This immediately produces a very characteristic white flocculent precipitate (Fig. 1.). This precipitate may also be obtained using a solution of lead subacetate (British Pharmacopoeia).

Gum arabic is resistant to acid hydrolysis. Hydrolysis to its constituent monomers may be achieved by reflux with concentrated sulphuric acid. Constituent monomers are identified by thin layer chromatography in order of increasing migration speed D-glucuronic acid, D-galactose, L-arabinose, L-rhamnose.

Though now entirely used as an ingredient in food or confectionary gum acacia found extensive use as an intravenous plasma expander during and in the years after World War I. Hurwitz (1917) first used gum arabic solutions clinically and Amberson (1936) was able to report seven papers recommending its use as a result of battlefield experience, and a further ten papers concerning its successful and uneventful use in over 1,800 cases of use in surgery, shock, obstetrics, neurosurgery and nephrotic disease. He concluded that intravenous gum acacia was in daily use throughout the world and that next to blood plasma it represented the most successful blood substitute then developed.

Despite this and other less critical advocacy of its use principally by its original investigators (Bayliss 1922) toxic effects of intravenous gum acacia were evident. Christie et al (1935) were able to demonstrate a reduced capacity oxygen carrying capacity for red cells after 15% gum acacia infusion, and Lucia and Brown (1934) and others recorded alterations in ESR secondary to red cell agglutination though this was felt by some to be only an in vitro phenomenon (Amberson 1936). Dick et al (1935) found reductions in haemoglobin and red cell volume in dogs after acacia infusion and concluded that these effects were not simply dilutional. More serious was the knowledge that acacia persisted in the body after infusion for long periods of time. Dick et al (1935) demonstrated hepatomegaly in all infants which received multiple transfusions for nephrosis along with suggestions of impaired liver synthesis manifest by reductions in serum proteins, especially albumin. The hepatomegaly was caused by acacia deposits. These changes were reversed when acacia treatment ceased. Studdiford (1937) found similar large accumulations in the liver of obstetric patients with eclampsia after acacia infusion and discovered

evidence of extensive acute fatty cell degeneration in those that died though in view of the severity of the underlying illness cause and effect were impossible to separate. Smalley et al (1945) administered gum arabic to dogs in doses greatly in excess of those administered to humans (x8-26) and despite confirming prolonged deposition in the liver indicated no "profound" hepatic damage had occurred as a result. Deposition of gum arabic was also shown to occur in spleen, kidney, lung, lymph nodes, and in bone marrow (Dick et al 1935). Small amounts were shown to be excreted in the urine (Studdiford 1937). Anaphylactic responses to gum arabic were believed to occur in humans (Lee 1922), though at the time the protein content (1.4%) of acacia was not appreciated and may have been the factor responsible for this phenomenon.

In view of the known accumulation of gum arabic within the body after transfusion and its success during slow low concentration infusions indicate that toxicity may have been dose dependent. Despite continued advocacy for its use (Johnson and Newman 1945), acacia solution ceased to be used widely in the 1940s probably as a consequence of the increased availability of blood, crystalloids and colloids.

Despite this widespread use in the early part of this century as an intravenous plasma expander and its other uses as an emulsifier and thickener in the food and confectionery industry, very little work has been carried regarding its metabolic fate in the gastrointestinal tract. Amberson (1937) summarised what was known or believed as a result of early experiments on gum arabic in the late 19th and early 20th century. Pearson (1818) claimed that gum arabic was an acceptable emergency ration for travellers in the desert regions of Africa though starch needed to be taken with it and quoted evidence that over one thousand men from an Abyssinian caravan survived for two months consuming only gum arabic during that time. Less anecdotal reports of the digestability of gum arabic however generally came to the conclusion that other than in herbivores gum arabic remained structurally intact during intestinal transit in mammals. Voit (1874) claimed

that gum arabic was partially degraded during intestinal transit on the grounds that only a proportion of the ingested material could be recovered from the faeces. Amberson also records reports of a laxative effect of gum arabic and, intriguingly, a Japanese study (Nakaschima, 1929) indicating that gum arabic might be attacked and degraded by intestinal bacteria or protozoa. More detailed examinations of the digestibility of gum arabic were conducted after Amberson's review though attention was then centred on its use as an intravenous agent. Monke (1941) administered high oral doses of gum arabic (c.34%) to young rats and concluded that as liver glycogen did not increase gum arabic must pass through the gastrointestinal tract intact. Booth et al (1949) administered 15% gum arabic orally to guinea pigs in a "basal synthetic diet", which reduced weight gain compared to a stock diet, and found that gum arabic would restore growth but not the products of gum arabic hydrolysis. He did not consider the potential caloric supply from gum arabic but did suggest it might alter bacterial flora. Roine et al (1949) found similar enhanced weight gain when gum arabic (15%) was added to a basal diet with added magnesium and potassium. O'Dell et al (1957) studied gum arabic metabolism in the guinea pig and concluded that it was 90% digested and that its bulking properties were superior to celluloflour. No details of gum arabic dose or the evidence for accepting a 90% digestibility were given. When gum arabic was consumed the absorption of calcium, magnesium and potassium was claimed to be increased by 10%, weight gain was increased, yet faecal output fell. No details of food intake were supplied as in previous studies making it impossible to exclude a hyperphagic response to the diet. Similar experiments in the rabbit suggested gum arabic utilisation (Hove and Herndon 1957). Gum arabic (20%) was administered for forty days and resulted in improved weight gain over that on a basal diet. In studies in rats Shue et al (1962) claimed that gum arabic (16%) was 80% digested and had a calorific value 75% that of sucrose. No information was given for the derivation of these figures but interestingly faecal nitrogen excretion increased with gum arabic administration. Booth et al (1963) fed gum arabic (15%) to rats for 62 days recording a mean weight gain of 224g insignificantly

different from control diet. No haematological effects were recorded and organ weights were said to be unchanged. Gum arabic (0.75 g.d) was calculated to be 71% undigested based on increases in dry faecal weight seven days after feeding a cathartic effect being explained on the presence of unaltered gum arabic in the faeces though no proof of this was provided. Gum arabic was calculated to have a caloric value 110-131% of corn starch at two dose levels (2.0 and 0.5g.d⁻¹) in rats fed for seven days. No information was provided on the methods used to calculate these figures (National Technical Information Service 1973). Recently evidence from rats fed elemental diet to which gum arabic 0-40% was added indicate extensive gum arabic degradation. With similar results for gums guar and tragacanth (Elsenhans et al 1981) Towle (1977) stated that gum arabic was unlikely to be degraded by the alimentary secretions of man and that gum arabic was probably degraded by bacteria. This was surprising in view of its structure as gums similar to gum arabic were somewhat resistant to bacterial attack. A similar assumption as to the digestibility of gum arabic has been arrived at by Adrian (1976) and by the US FDA (1974) who accept digestion by herbivores and a degree of digestion by omnivorous species including man. In vitro evidence supports the bacterial nature of gum arabic degradation. Salyers (1979) indicated a wide variety of bacteria in human faeces are capable of the fermentation of complex polysaccharides and fermentation of gum arabic by Bifidobacteria has been demonstrated (Salyers et al 1978). Information, therefore, on the digestibility of gum arabic in animals is indefinite though the indications are of significant degradation by bacteria. In man there is no evidence derived from direct administration of gum arabic but again assumptions have been made by extrapolation from animal data and from in vitro work. In vitro evidence suggests that gum arabic has no teratogenic effects in test animals and has no mutagenic or carcinogenic effects on rats as assessed by a host mediated assay, a cytogenic assay, and in a dominant lethal gene test (Stanford Research Institute 1972). It has been concluded that gum arabic probably exerts no adverse effects on humans when consumed in standard quantities but that the effects of significant increases in consumption are unknown (Federal Drug Administration 1974).

The definition of dietary fibre.

Dietary fibre is a misleading term suggesting as it does a fibrous or strand-like structure which many substances classed as dietary fibre clearly do not have. Alternative terminology such as "plantix" (Spillar) or dietary fibre complex (Trowel 1976) has been suggested but has not been widely adopted and dietary fibre (D.F.) remains in common use. Trowel (1974) defined D.F. as the remnants of plant cells resistant to the alimentary enzymes of man. This definition proved restrictive, excluding storage polysaccharides, mucillages, and algal polysaccharides. Recognition of the metabolic properties that such complex polysaccharides might display during intestinal transit and their potential therapeutic benefits indicated that they should be included with D.F. despite their relatively small effects on colonic performance and faecal characteristics. Nevertheless, difficulties arise with their inclusion in a definition based on the structural concept of the cell wall. Other non plant polysaccharides such as connective tissue polysaccharide, mucus glycoprotein, and mucopolysaccharide are untouched by endogenous secretion and might therefore be included. Furthermore the inclusion of lignin, an aromatic non-polysaccharide compound, suggests that other non polysaccharides such as protein, trace minerals, enzymes, and vitamins found in the cell wall might be included as D.F. (Trowel 1977). An alternative definition "the plant polysaccharides and lignin that are resistant to the digestive enzymes of man" (Trowel et al 1976) is more satisfactory and allows for the inclusion of storage polysaccharides and the exudate gums including gum acacia. More precision is added by the term non starch polysaccharides (N.S.P.) which may further be subdivided into cellulosic and non-cellulosic polysaccharide (Cummings 1981). Definitions in terms of nutritional availability have fallen from favour. Originally suggested by Van Soest (1966) they introduced confusion. Knowledge of the variable degradation of D.F. by colonic bacteria makes such terms as non nutritive residue and indigestible residue as meaningless. Criticisms of these terms have been accepted by their original proponents (Van Soest and Robertson 1977). Similarly definitions based on characterisations of botanical structure are of little use in D.F. research since tedious and complex procedures are required which are impractical for routine physiological research (Southgate

1977).

A satisfactory definition of D.F. will therefore encompass a wide variety of complex compounds. A problem then exists in proposing an all inclusive term with the resulting chemical problem of separating and identifying the vast number of compounds that will be included. Chemical expressions of fibre content remain the most generally used form of analysis. Original expressions of crude fibre, though still widely employed, are inappropriate for nutritional work. Sequential extraction by acid and alkali result in the extensive loss of lignin (50-90%), hemicellulose (85%) and cellulose (0.5%) (Van Soest 1978). Other techniques use chemical or enzymatic techniques, but each category identifies different substances. Acid detergent fibre includes cellulose and lignin as well as contaminants such as animal skin hair, silica and plastic but excludes the hemicellulose component of D.F. (Van Soest 1978). Neutral detergent fibre (N.D.F.) expressed values for cellulose, hemicellulose and lignin though cutin, minerals and protein also remain. This method has found much favour although like other methods it does not estimate water soluble polysaccharides (Southgate 1977) and may result in over estimates due to the presence of unabsorbed starch (Brauer et al 1981). Enzymatic analyses developed by Hellendoorn et al (1975) and Engelyst (1981) produce results broadly similar to neutral detergent fibre though bacterial cell walls are preserved to a greater extent and thus result in higher values (Southgate 1977). A more exacting method described Southgate (1969) involving separate hydrolysis of non cellulosic polysaccharide and cellulose to component sugars after extraction of starch has the advantage of greater precision and measures both water soluble and insoluble polysaccharide by extraction using alcohol.

Broadly speaking chemical expressions of fibre content will measure one of the following categories: 1. plant cell walls - essentially cellulose, hemicellulose and lignin; 2. substances resistant to the endogenous secretions of man which include non structural polysaccharides such as storage polysaccharides,

mucillages and exudate gums and pectin; 3. all substances provided in food untouched by endogenous secretions which will include non plant polysaccharides, many synthetic substances and degradation products from cooking such as Maillard reactions which have little to do with the concept of dietary fibre but may influence measurements of its action (Van Soest and Robertson 1977). The second category has been largely accepted and non structural polysaccharides such as gum arabic, gum guar and pectin are reasonably included as part of dietary fibre (Southgate 1977). (Fig.1).

Source		Major Groupings	Principal Structural Types
Structural material of the plant cell wall	(Non-cellulosic polysaccharide	Galacturonans
	(Xylans - arabino/
	(glucurono
	(Cellulose	Manuans - gluco/
	(galacto
	(Galactans, arabino
	(B-D-glucan
Non-structural materials either found naturally or used as food additives	(Lignin	Aromatic Polymer
	(Pectin	Galacturonans
	(Great variety
	(including arabino-
	(Gums	xylans, gluco- and
	(galacto-mannans,
	(sulphated galactans
	(Mucillages	
	(Glucurono-
	(Algal polysaccha-	mannuronans
	(rides	
	(Modified celluloses	Esters
	(Modified starches	Esters

The Components of Dietary Fibre (from Southgate 1977).

The heterogeneous nature of dietary fibre makes for great difficulties in analysis, and adequate methods are not available to characterise individual sources of D.F. on a routine basis (Cummings 1982).

Evidence for the metabolic fate of fibre.

Herbivorous animals derive the majority of their energy requirements from the degradation of dietary fibre (D.F.) (Bergman et al 1965). It is now generally agreed that dietary fibre is also degraded to a variable degree, during its passage through the GI tract of omnivorous species and is not an inert bulking substance. In studies of digestibility, methodological problems often make assessment of data and comparison with other studies difficult. The unacceptability of crude fibre as a means of estimating D.F. intake has been referred to earlier. Acid detergent fibre dominates hemicellulose during analysis. Neutral detergent fibre, probably the most universally employed analytical method, may underestimate fibre degradation by approximately 6% unless unabsorbed starch is removed enzymatically as a preliminary (Brauer et al 1981). Even then this measurement will still not estimate water soluble polysaccharides (Cummings 1981) which are substantially or completely degraded during intestinal transit. Nevertheless since Williams and Olmstedt (1936i) demonstrated, using relatively inaccurate methods, the variable disappearance of D.F. from a variety of vegetables and cereals, many studies have provided evidence of dietary fibre degradation in animals and man. Cummings (1981) has estimated that approximately 15g dietary fibre will be degraded daily by subjects on a standard Western diet. Degradation of any D.F. source is dependent upon its constituents. On a mixed fibre diet approximately 75% fibre degradation has been observed using an N.D.F. method corrected (Brauer et al 1981) or uncorrected (Calloway and Kretsch 1978) for starch polysaccharide. Addition of 45g of wheat bran to such a mixed fibre diet resulted in a reduction of N.D.F. digestion to 50% (Calloway and Kretsch 1978) reflecting the generally low digestibility of bran which has variously been reported as between 27%-50% (Saunders 1978, Heller et al 1980, Stephen and Cumming

1980ii). The individual polysaccharide components of cereal fibre, cellulose and hemicellulose are digested to a varying degree. Cellulose digestibility is generally regarded as between 40%-50% (Cummings 1981, Prynne and Southgate 1979) though much reduced levels have been recorded of 15-44% (Southgate and Durnin 1970) and 6-23% (Heller et al 1980). These very variable figures illustrate methodological differences since the low digestibilities quoted in the former studies were probably strongly influenced by the inclusion of lignin in the calculations (Prynne and Southgate 1979). In contrast Carrier et al (1982) observed no digestion of isotopically labelled cellulose fibres during intestinal transit though extensive chemical modification during labelling probably accounted for its indigestibility. Hemicelluloses in contrast are more digestible than cellulose as early experiments with vegetable and cereal fibres demonstrated (Williams and Olmstedt 1936i). Cummings (1981) estimates that 80% of hemicelluloses will be degraded during intestinal transit.

D.F. of vegetable origin (cabbage) has been shown to be highly degraded (92%) in comparison to wheat bran (27%) (Stephen and Cummings 1980ii). In general water soluble polysaccharides are highly degraded during intestinal transit. Subjects given 25 g.d^{-1} non cellulosic polysaccharide in the form of ispaghula husk showed a mean digestibility of 83% though the figure was highly variable between subjects (Prynne and Southgate 1979). Cummings et al (1979iii) studied five male subjects who consumed 36g pectin daily. High levels of cellulosic and non cellulosic polysaccharide digestion were noted. Non cellulosic uronic acid excretion was not increased and pectin digestibility estimated at 99%. Non cellulosic polysaccharides are therefore extensively degraded during intestinal transit though the presence of cellulosic polysaccharide or lignin inhibit this degradation (Cummings 1981i). Similar findings have been recorded in studies in the rat. Cellulose digestibility is similar to that in humans (Hove and King 1979). Nyman and Asp (1981) using an enzymatic digestion method for fibre analysis calculated digestibility of bran, guar and sugar beet pulp to be 37%, 100%, 74% respectively. In this study pectin digestibility

was estimated at 75-80% which is lower than recorded in human studies though differences in methodology may explain these. Hove and King (1979) recorded similarly low pectin digestibility (71-84%) using a method for estimation which has not been traced for this review.

The large intestine is the major site of polysaccharide fermentation in monogastric species. High concentrations of V.F.A. in the caecum and colon of the horse (Argenzio et al 1974), the pig (Argenzio and Southworth 1975) and the rat (Elsden et al 1946; Remesy and Demigne 1976) suggest that these organs are the prominent sites of D.F. fermentation. In man the evidence is less direct. Vercellotti et al (1978) in a study of fresh cadavers demonstrated significantly greater (x4) concentrations of high molecular weight carbohydrate in the ileum than the colon. Cummings (1981) has suggested that a greater proportion of fibre breakdown in the colon occurs in man due to the smaller relative size of the caecum when compared to animals such as the rat. Excretion of V.F.A. decreases after colonic resection (Cummings et al 1973), though the subjects used to identify this all had post resectional diarrhoea. Though by definition untouched by endogenous alimentary secretions some evidence suggests the dietary non starch polysaccharide (N.S.P.) may be partially degraded during passage through the upper alimentary tract. Keretz (1940) noted no digestion of pectin in salivary gastric or small intestinal juices in vitro though a faecal suspension produced rapid fermentation. Sandberg et al (1981) claimed to have found minor degradation of arabinose and xylose elements of bran after incubation with human gastric juice but no further details were available. Xylose has also been reported to be present in the small bowel content of fresh cadavers suggesting the possibility of cleavage of monomers or oligosaccharides from N.S.P. in the human stomach or small intestine (Vercellotti et al 1978). Holloway et al (1978) studied patients with ileostomies. Ileostomy effluent was collected 8-10 days after commencing a constant mixed diet of constant proportions. Cellulose and hemicellulose degradation were reported as 15.5% and 72.5% respectively based on N.D.F. in the ileal effluent. Sandberg et al

(1981) carried out more controlled studies feeding bran 16 g.d^{-1} to ileostomists and found digestibility of cellulose and hemicellulose to be approximately 0-25%, and claimed that methodological defects such as delays in emptying ileostomy bags may have affected the previous study by allowing extracorporeal N.S.P. fermentation. Nevertheless delays of up to two hours occurred in Sandberg's study which could still account for some of the N.S.P. digestion. Furthermore surgery may have resulted in adhesions with subsequent areas of adhesions and intestinal stasis, which may result in sites of small intestinal fermentation (Chernov 1972). Low concentrations of V.F.A. ($1-3 \text{ mmol.l}^{-1}$) suggest that little fermentation of fibre normally occurs during small intestinal transit (Cummings 1975) though Schmitt (1976) has demonstrated that VFAs present in the human jejunum will be rapidly absorbed. Further studies on the fate of fibre in the upper gastro intestinal tract have been suggested (Cummings 1975).

Many factors influence the metabolic fate of D.F. Digestibility of fibre varies widely between individual subjects (Stephen and Cummings 1980ii). Southgate and Durnin (1970) recorded cellulose digestibility between 15 and 44%. Prynne and Southgate (1979) studied ispaghula (25 g.d^{-1} N.C.P.) digestion and found great variability in digestion of hexose and pentose though less so for uronic acid between subjects. Pentose digestibility varied between 55-100%. Heller et al (1980) have suggested that cellulosic fractions are particularly variable though their study was marred by a major alteration in caloric intake during the study necessitated because of weight loss in the subjects. Williams and Olmstedt (1936) demonstrated great differences in cellulose digestion between sources of D.F. Thus 7% of cellulose in cellufLOUR, 29% in bran and 67% in carrot were digested. Refinement of fibre results in increased digestibility. Within the limitations of their study Heller et al (1980) have indicated the bran digestibility increased with a reduction in particle size which also markedly increased cellulose digestion presumably by increasing bacterial availability. Purification of cellulose, thus increasing its crystallinity, decreases digestibility

of cellulose (Van Soest 1973) probably by reducing its surface area (Cummings 1981).

Lignin, an aromatic non polysaccharide usually considered with D.F., is poorly digested and its presence will in general reduce the digestibility of fibre (Williams and Olmstedt 1936i; Van Soest 1973) by preventing enzymic access to the carbohydrate moiety (Gordon 1978). In ruminant studies lignin extraction renders D.F. more digestible (Dekker et al 1973). The variable digestion of bran may be in part due to the known variable lignification of bran (Southgate and Van Soest 1978).

Contamination of D.F. with substances such as silica and cutin will reduce the digestibility of bran (Van Soest 1973). Admixture of sources of D.F. also seems to affect digestibility thus Prynne and Southgate (1979) noted parallel reductions in the digestion of ispaghula and cellulose when administered together.

The digestibility of water soluble N.S.P. has already been mentioned (Cummings et al 1979iii; Prynne and Southgate 1979) and this probably relates to ease of bacterial access in contrast to the hydrophobic nature of highly lignified D.F. such as bran. The evidence in rats of the digestion of guar gum and the limited evidence for gum arabic digestion confirms the importance of water solubility to digestion.

The rate of transit of intestinal content may also affect digestibility. Cummings (1982) demonstrated marked reductions in the digestion of cellulose and to a lesser extent pentose when intestinal transit time dropped below 50 hours. Hexose was less affected and uronic little affected by these alterations. Southgate and Durnin (1970) observed greater cellulose digestion in elderly patients and suggested that this could be due to an increased transit time though this was not measured. Certainly in the ruminant cellulose digestion will continue for up to 48 hours (Van Soest 1973). However Brauer et al (1981) were unable to confirm any difference in N.D.F.

digestibility between elderly and young subjects in a study specifically designed to examine this thesis. When transit time becomes pathologically increased fermentation may be affected. Cummings et al (1973) detected low levels of faecal V.F.A. in patients with severe post resectional diarrhoea.

As in herbivores bacterial fermentation results in destruction of simple and complex carbohydrate molecules which reach the large bowel. Bacteria have been demonstrated in intimate contact with fibre in human faeces (Williams et al 1978) and many species of human faecal bacteria will ferment polysaccharides from D.F. in vitro. Salyers et al (1978) studied fermentation patterns of 25 species of bacteria on 20 complex polysaccharides. *Bacteroides* strains fermented 7 polysaccharides, and bifidobacteria 7, including gums arabic, guar, and tragacanth. Cellulose, non cellulosic glucans, pectins, mucopolysaccharides and mucin glycoproteins all have anaerobic bacteria capable of their fermentation in vitro (Bryant 1978; Salyers 1979; Salyers et al 1977). *Bacteroides fragilis*, *Ruminococcus bromii*, and *Peptostreptococcus productus* are the most numerous faecal bacteria and are presumed to be the predominant fermentors of D.F. in the human (Bryant 1974).

Pathways of fermentation in man are similar to those of ruminants and equations have been derived for humans which approximate to those applicable to ruminants (Miller and Wolin 1979). These equations however assume methane production and the utilisation of hexose as an energy source and do not fully explain fermentation in non methane producers and for differing energy substrate. Salyers et al (1978) emphasised that bacterial fermentation is inducible and thus previous dietary intake may be important in assessing in vitro bacterial behaviour or when measuring in vivo fibre degradation.

The constancy of bacterial faecal flora has been noted by a number of authors. Furthermore the faecal flora is representative of the proximal colonic flora (Moore et al 1978).

Walters et al (1975) noted no changes in faecal flora after 12 weeks ingestion of wheat, bran, or bagasse, and similar findings after consumption guar, pectin, banana, and wheat fibre have been recorded (Drasar and Jenkins 1976; Drasar et al 1976). Fuchs et al (1976) demonstrated increased 24h. excretion of anaerobes but no significant changes in other flora other than an increased anaerobe:aerobe ratio. When fibre is removed from the diet by administration of a defined or elemental diet no great alterations in bacterial flora occur. (Burnside 1978, Crowther et al 1973). Burnside and Cohn (1975) demonstrated stable aerobic and anaerobic concentrations in the faeces after 7 days on an elemental diet despite a reduction in daily faecal weight by 70%. Winitz et al (1970i) suggested however that marked reductions in concentrations of aerobes and anaerobes could be detected shortly after commencing an elemental diet. The reasons for this discrepancy are uncertain. Enemata were administered at the start of Winitz' study and may have affected sampling of faeces though mechanical cleansing of the colon is generally regarded to have no effect on bacterial concentrations (Burnside and Cohn 1969). Gall (1968) found changes in proportions of anaerobic species but no alteration of concentrations on elemental diet. Despite their apparent constancy in other situations antibiotics markedly reduce bacterial numbers (Burnside 1978).

Bacterial fermentation of polysaccharide is an anaerobic process. Hexose is metabolised to pyruvate via the Embden Meyerhoff pathway and partly through the pentose pathway which represents the route for pentose metabolism. Subsequent metabolism gives rise to the end products: H_2 , CH_4 , H_2O and the VFA acetate, propionate, butyrate, lactate may also be an end product. In health lactate concentrations are very low. Breast fed babies have appreciable concentrations where compared to those artificially fed (Weijers and De Maker 1963). Lactate production increases when large quantities of undigested carbohydrate enter the colon as a result of infective diarrhoea or disaccharidase deficiency (Torres-Pinedo et al 1966) or after administration of

large quantities (150 g.d^{-1}) of legumes (Hellendoorn 1978).

Volatile fatty acids (V.F.A.), acetate (Ac), propionate (Pro), butyrate (but), isobutyrate (isobut), valerate (val), isovalerate (isoval) are found in the faeces and intestinal contents of numerous species, the rat (Remesy and Demigne 1976), sheep (Bergman et al 1965), horse (Argenzio et al 1974), pig (Argenzio and Southworth 1975) and dog (Stevens 1978) as well as man. Yang et al (1970) recorded that V.F.A. had been demonstrated in the caeca of 7 monogastric animals. Concentrations in the large intestine were equal to or greater than those in ruminants (Stephens 1978). Cummings (1981) has noted the broad similarity in the molar ratios of the three principle V.F.A. (ac, pro, but) in the intestinal content of ruminants and monogastric species.

V.F.A. production arises from the fermentation of polysaccharide from D.F., mucus glycoprotein, mucopolysaccharide from intestinal secretions, effete epithelial cells, and from connective tissue and ground substance. Thus dogs maintained on a low fibre carnivorous diet continue to produce V.F.A. (Banta et al 1979).

Fasting reduces concentrations of V.F.A. in the rat caecum (Remesy and Demigne 1976) and concentration levels fall in an exponential fashion as fasting continues (Yang et al 1970). Similar, cyclical, variation in V.F.A. concentration have been noted in the pig (Argenzio and Southworth 1975). V.F.A. concentration in swallowed dialysis bags fell in subjects who consumed only distilled water and methyl cellulose (60g) for four days (Rubenstein et al 1969). In the same study the administration of a triple antibiotic solution, as anticipated, produced marked reductions in dialysable V.F.A. particularly in the acetate and butyrate fractions. Gompertz et al (1973) studied leukemic patients maintained on wide spectrum antibiotics and in bacteriological isolation during treatment. Faecal V.F.A. concentrations fell markedly and returned rapidly to normal on removal of the patient from isolation and after the cessation of antibiotic.

The rate of intestinal transit may alter V.F.A. production. Spillar et al (1980) were unable to demonstrate a relationship between transit time and V.F.A. concentrations or output after feeding pectin or cellulose. However in studies of patients with severe post resectional diarrhoea (faecal weight 664-1528 g.d⁻¹) V.F.A. concentrations fell with increasing severity of diarrhoea as a result, it was suggested, of incomplete digestion of fibre (Cummings et al 1973). Faecal water content was not mentioned in this study and the effect may have been dilutional consequent upon impaired water absorption though sodium and chloride concentrations tended to rise with worsening diarrhoea. In the pig constant concentrations of V.F.A. along the length of the large bowel have been attributed to its rapid transit time (Argenzio and Southworth 1975).

Conflicting evidence exists as to the effect of diet on V.F.A. production. Williams and Olmstedt (1936i) measured V.F.A. output in subjects fed cellufLOUR, alfalfa, cotton seed mill, peas, carrots, wheat, bran and cabbage and noted respective increases in V.F.A. production for each of the D.F. sources. They also noted that V.F.A. concentrations were related to the apparent digestibility of the D.F. in the diet, though the limitations of the analytical methods in this study have been mentioned. Ehle et al (1982) demonstrated greater concentrations of faecal V.F.A. after cabbage than after cellulose ingestion. This difference, present after six weeks, was absent after two weeks though digestibility was not altered with time. Hellendoorn (1978) reported highly variable alterations in V.F.A. excretion 3-4 days after administering a large dose of beans (150g). Lactate levels rose in this study perhaps reflecting preferential lactate production in the presence of large amounts of carbohydrate in the caecum. Cummings et al (1979ii; 1976) reported no changes in faecal V.F.A. concentrations after the addition of 30g or 45g cereal fibre to the diet, though V.F.A. output increased when the higher dose of fibre was consumed. Pectin in relatively low dosage (6 g.d⁻¹) increased faecal V.F.A. concentrations and daily V.F.A. output when administered to subjects known to have slow (>3 days) intestinal transit (Spillar et al

1980). In the same study cellulose (14 g.d^{-1}) increased daily V.F.A. excretion but did not alter concentrations. Studies in monogastric animals have provided further information on diet and its effect on V.F.A. levels. Remesy and Demigne (1976) demonstrated low concentrations of V.F.A. in the distal stomach duodenum and ileum with intermediate levels in the proximal stomach. Maximal concentrations were found in the caecum, the levels being constant at two points within it. Though slightly lower than caecal concentrations, colonic and faecal levels were little different. Thomson et al (1982) added pectin (50%) to a high fat (17%) diet and noted substantial increases in caecal V.F.A. concentrations in the rat, acetate increasing by 50%. A linear increase in caecal V.F.A. content has been demonstrated in rats on high or low protein diets with graded increases (0-10%) in pectin ($r=0.68$ and 0.89 respectively for each diet) (Hove and King 1979). In the same study cellulose (0-20%) produced increases in caecal V.F.A. though these were not as great or as linear as with pectin ($r=0.50$ and 0.44). Modest increases in caecal V.F.A. content and faecal V.F.A. output in rats fed 0-10% cellulose have been demonstrated by Yang et al (1969). Cellulose appeared highly digestible, 80-90% conversion to V.F.A. being estimated. In spite of apparently consistent effects in the rat results in other animal models have produced conflicting results. Pigs when fed a high cellulose diet produced concentrations of colonic V.F.A. lower than when on a regular diet (Argenzio and Southworth 1975) though these concentrations were high when compared to colonic concentrations in ruminants. Banta et al (1979) compared the effects of a cereal based diet with a canned meat diet on V.F.A. concentrations in the canine alimentary tract. Caecal pH fell, colonic and caecal lactate concentrations increased but no differences arose between V.F.A. concentrations on the two diets.

V.F.A. are the major energy source for ruminant species, 70-80% of their energy requirements being provided (Bergman et al 1965). Absorption of V.F.A. from the caecum colon and rectum has been demonstrated in a number of monogastric species including man.

It represents the major fate of V.F.A. in man with smaller amounts excreted in the faeces or metabolised to produce H_2 , CH_4 , CO_2 , and water (Cummings 1975). Absorption of V.F.A. has been demonstrated by intraluminal injections of radiolabelled V.F.A. into the rat caecum and colon (Yang et al 1970; Bond and Levitt 1976) and by estimation of portosystemic arteriovenous differences in V.F.A. concentrations (Remesy and Demigne 1976; Buckley and Williamson 1977). Acetate is rapidly absorbed after caecal injection, 55% of ^{14}C label appearing in the breath as $^{14}CO_2$ within $2\frac{1}{2}$ hours (Yang et al 1970). Ruppín et al (1980) have confirmed rapid absorption of propionate acetate and butyrate at equal rates after caecal instillation through enteric tubes. McNeil et al (1978) have also found rectal absorption of V.F.A. from dialysis bags and that absorption rates are similar in the proximal, transverse and distal colon (McNeil et al 1978). In a study of a single colostomist with a mucus fistula Dawson et al (1964) claimed rapid absorption of V.F.A. using a washout technique but suggested that absorption rate was proportional to V.F.A. molecular chain length.

In humans V.F.A. absorption from the large intestine is accompanied by increased intraluminal bicarbonate concentrations and a reduction in intraluminal pCO_2 (Ruppín et al 1980; McNeil et al 1978) and these changes are accompanied by a rise in intraluminal pH. Sodium absorption is increased in the presence of V.F.A. absorption. Whether V.F.A. is absorbed ionised or unionised or whether active transport occurs is uncertain. Rectal absorption is unaffected by a reduction in intraluminal pH (McNeil et al 1978) suggesting a role for absorption in the ionic form, and Ruppín et al (1980) have proposed that 40% of absorption occurs as ionised V.F.A. Intestinal mucosa is, however, relatively impermeable to the ionic form (Cummings 1981). Though exchange of V.F.A. for intracellular bicarbonate would explain pH and bicarbonate alterations in man it now seems more likely that mucosal carbonic anhydrase results in intraluminal CO_2 hydration. Protonation of the acid produces an increased pH and the absorption of the acid in unionised form (Ruppín et al 1980; Argenzio et al 1977; McNeil et al 1978).

A small proportion of V.F.A. is utilised by the intestinal mucosa itself. Remesy and Demigne (1976) reported the utilisation of 12% of caecal butyrate by the caecal wall of the rat but found no consumption by the colonic wall quoting similar results from other French workers. Butyrate has since been shown to be preferentially used as an energy source by isolated colonocytes though this property was more marked in colonocytes of distal colonic origin (Roediger 1980).

It seems V.F.A. absorption contributes to energy balance in monogastric animals though to a much lesser degree than in ruminants. Caecal absorption by the rat represents 9-10% of total energy intake. On the assumption that 20g fibre is digested by man per day, energy provision would be approximately 224 kJ.d^{-1} . Much higher levels of energy ($600-700 \text{ kJ.d}^{-1}$) might be available from calculations based on bacterial proliferation (Cummings 1981). Clearly energy production from V.F.A. could be substantial in populations consuming large quantities of D.F.

Bacterial fermentation of unabsorbed carbohydrate also results in the production of hydrogen and methane which, after absorption into the portal circulation, are excreted from the lungs in measurable amounts. Hydrogen production is produced by aerobic and to a much greater extent anaerobic metabolism (Bond and Levitt 1978), and though the precise mechanisms of methane production in man is unknown it may occur entirely as a result of anaerobic bacterial activity (Bond et al 1971) (McKay et al 1981). Clostridia are the major producers of hydrogen, while gram negative enteric bacteria such as E.Coli have little activity in vitro (Steggerda 1968). A methanogenic bacterium, *methanobacterium ruminococcus* has been isolated from human faeces (Nottingham and Hungate 1968).

In health hydrogen production, universal in adult subjects, almost entirely occurs in the large bowel with less than 1% arising from the small bowel (Levitt and Ingelfinger 1968). An antegrade intestinal intubation study to the splenic

flexure in a single patient suggests that just over 50% of total hydrogen production occurs proximal to the splenic flexure with the remainder arising from fermentation more distally (Levitt and Ingelfinger 1968). If the small intestine becomes colonised with anaerobic bacteria production of hydrogen may result (Levitt 1969) and is abolished when antibiotics are administered (Steggerda 1968; Gilat et al 1978). After production approximately 14-20% of intraluminal colonic hydrogen will be absorbed and excreted in exhaled breath (Levitt 1969). Rises in intraluminal methane and hydrogen have been reflected in increased excretion in the breath in most studies (Levitt 1972; Calloway and Murphy 1968). Marthinsen and Fleming (1982) in a study using indwelling rectal catheters could not correlate flatus and breath hydrogen levels but found such a correlation for methane.

The unabsorbed oligosaccharides raffinose, stacchiose and lactulose result in increased breath hydrogen excretion either singly (Tadesse and Eastwood 1978) or for the former two as beans (Calloway and Murphy 1968). Absorbable oligosaccharide such as lactose will if injected into the caecum (Levitt and Ingelfinger 1968) or in the presence of small bowel anaerobic colonisation result in increased breath hydrogen excretion. Polysaccharides appear to result in less marked responses. Hemicellulose (10-20g) increased breath hydrogen excretion after a single dose taken by fasted subjects, but cellulose pectin and lignin failed to do so when taken in the same manner (Tadesse and Eastwood 1978). Raw carrot fibre (6 g.d^{-1}) taken for 3 weeks caused increased breath hydrogen excretion after 10 days on the diet (Robertson et al 1979). Bond and Levitt (1978) studied subjects to whom 10, 20 or 30 g.d^{-1} bran was administered and found increased quantities of H_2 excreted daily whilst taking the two highest doses ($0.5, 8.0, 12.0 \text{ ml.H}_2 \text{ g.d}^{-1}$ respectively) no information was given concerning hydrogen excretion from unsupplemented controls however. Marthinsen and Fleming (1982) studied the effects of xylan, pectin, and cellulose (0.5 g.kg^{-1}) and corn bran (isocaloric replacement, 80%, for cornstarch) added to a fibre free basal diet. Flatus and breath gases were collected. Pectin and xylan produced significantly greater breath hydrogen

excretion than cellulose or corn bran but none of the diets achieved levels of excretion higher than those measured on a baseline fibre free diet.

In contrast to hydrogen, methane production is not universal among humans. Between 30 and 60% of the adult population are excretors (Bond et al 1971, Pitt et al 1980; Calloway and Murphy 1968; McKay et al 1981). Pitt et al (1980) found a higher proportion of female producers though this was due to high numbers of producers in European born Caucasian women. Oriental and Indian subjects had lower proportions of producers than Blacks or Caucasians. Unlike hydrogen production which can be detected soon after birth, methane only becomes detectable after 6 months (Bond et al 1971) though methane producing status remains constant thereafter.

Hydrogen production varies through the day with a fall in the morning and a rise in the afternoon (Tadesse and Eastwood 1978; Marthinsen and Fleming 1982) but methane excretion is little affected by time of day or fasting. In humans breath methane levels are less affected by diet than hydrogen (Tadesse and Eastwood 1978) in contrast to its vigorous production in the rumen which is governed by dietary substrate intake (Czerkawski and Breckenridge 1969). McKay et al (1981) in a population study in Edinburgh found significant correlations between pentose and lignin intake and methane producing status although lignin intake had previously been shown to have no effect on methane excretion after acute ingestion (Tadesse and Eastwood 1978). Ingestion of the pentose monomers D+xylose and L+arabinose resulted in increased breath methane excretion by producers but consumption of complex polysaccharides rich in pentose such as xylan oranges, bran, carrot and apple failed to influence methane excretion (McKay et al 1981) when administered in single doses.

Effects of dietary fibre upon colonic function.

Dietary fibre has marked effects on colonic function whereas other components of the diet do not. Cummings et al (1978i) studied faecal characteristics in male subjects whilst on

a low fat (62 g.d^{-1}) or high fat (152 g.d^{-1}) diet and found no alterations in transit time, stool weight, or bowel habit though bile and fatty acids were increased in the stool. In a study of 4 subjects the effect of increasing protein intake from 62.7 to 136 g.d^{-1} was examined and again no alterations in stool characteristics occurred (Cummings et al 1979i). In the same study addition of wheat fibre (31g) to the diet resulted in a significant increase (160%) in wet weight of stool. This profound effect on stool weight caused by cereal fibre has been confirmed by many other studies in healthy subjects (Kirwan et al 1974; Eastwood et al 1973; Cummings et al 1976; Cummings et al 1978ii; Stephen and Cummings 1979; Paylor 1973; Heller et al 1980; Findlay et al 1974; Judd and Truswell 1981; Kirby et al 1981; Jenkins et al 1975; Rheingold et al 1976). Patients with diverticular disease show similar responses (Smith et al 1981; Tarpila et al 1978; Brodribb and Humphries 1976) though Findlay et al (1974) found that bran (20 g.d^{-1}) increased faecal wet weight less ($+17 \text{ g.d}$) in patients with diverticular disease than in apparently healthy subjects ($+63 \text{ g.d}$). Cereal fibre is therefore highly efficient at inducing increases in faecal weight. Vegetable fibre and fruit are less so. Cummings et al (1978ii) administered bran, cabbage, carrot and apple to healthy subjects and noted increases in daily faecal weight of 127%, 69%, 59% and 40% respectively. They calculated that to double faecal weight would require daily consumption of each respectively 47g, 775g, 681g and 1447g emphasising the efficiency of bran in this respect. Least effective are highly degraded water soluble fibre such as gum guar and pectin. Thus 20 g.d^{-1} guar resulted only in a 20% increase in wet weight (Cummings et al 1978ii). Pectin (36 g.d^{-1}) increased wet weight by 33% in one study (Cummings et al 1979iii) and by 24% in a study (pectin 12 g.d^{-1}) of subjects with slow intestinal transit times (Durrington et al 1976). In a further study pectin in considerably lower dosage (6 g.d^{-1}) insignificantly reduced faecal wet weight while cellulose (14 g.d^{-1}) resulted in an increase of 34 g.d^{-1} faecal excretion (Spillar et al 1980). In general cereal fibre will increase faecal weight by approximately 6 g.g^{-1} D.F. consumed though Spillar (1982) suggests that subjects with very

low or high initial faecal weight do not respond "normally" to dietary fibre and must be excluded if this figure is to be reached. Epidemiological evidence supports the findings of these experiments and vegetarian populations excrete more bulky faeces than do their omnivorous counterparts (Burkitt 1975; Burkitt et al 1972), though neither of these groups approach the faecal bulk of rural Africans in whom faecal weight may be in excess of 450g.d^{-1} (Burkitt et al 1972).

Alterations in faecal weight are accompanied by changes in intestinal transit. Burkitt et al (1972); Kirwan et al (1974) demonstrated the curvilinear relationship between dietary fibre intake and stool weight. The major part of intestinal transit is spent in the colon. In general transit time is reduced when cereal D.F. intake is increased. Thus Paylor et al (1975) noted a 27% reduction in mouth to anus transit after bran (20g.d^{-1}). Similar reductions in transit time after cereal bran have been recorded by others (Cummings et al 1976; Kirwan et al 1974; Smith et al 1981) but in two studies bran failed significantly to alter transit time (Eastwood et al 1973; Findlay et al 1974) though in the latter transit time reduced significantly in patients with symptomatic diverticular disease. In contrast pectin has been shown to have little effect on mouth to anus transit (Cummings et al 1979iii; Durrington et al 1976) though at a low dosage (6g.d^{-1}) Spillar (1980) was able to record an increase in transit time. While gums and pectin have little effect on total intestinal transit they slow small intestinal transit. Jenkins et al (1978) administered gums guar and tragacanth, methyl cellulose and pectin (20g) and bran (40g) to normal volunteers. Using a lactulose breath hydrogen response they demonstrated progressive retardation of the response by pectin, tragacanth and guar, and an acceleration by bran. Interestingly, if mouth to anus transit is initially rapid then addition of cereal fibre will lengthen transit time. Harvey et al (1973) studied 20 subjects who consumed a high fibre diet with 30g.d^{-1} added bran. Eight subjects decreased transit time and all had initial transits of three days or more. Five subjects increased transit time and all had transits of 24 hours or less

before bran. Brodribb and Humphreys (1976) in a long term study of patients with diverticular disease treated by bran (24g.d^{-1}) for six months found similar responses in transit time.

An important effect of cereal fibre is its ability to lower intraluminal colonic pressure. Kirwan et al (1974) and Findlay et al (1974) both demonstrated reductions in intraluminal pressure after bran (20g.d^{-1}). Brodribb and Humphreys (1976) also demonstrated reductions in colonic high pressure waves in patients with diverticular disease after treatment with bran (24g.d^{-1}) for six months. Conversely ispaghula, a polysaccharide highly degraded during intestinal transit, resulted in an increase in intraluminal pressure when compared to the effect of bran (Eastwood et al 1978), increasing the frequency of high amplitude pressure waves. Methyl cellulose (Celevac) has a similar pressure lowering effect to bran (Hodgson 1972).

These responses to fibre may be influenced by other factors. Thus cooking bran reduces its ability to increase faecal weight and decrease transit time in healthy volunteers (Wyman et al 1976). Similarly Kirwan et al (1974) have demonstrated that coarse bran (20g.d^{-1}) will significantly reduce post stimulation motility index and transit time while finely ground bran does not. Brodribb and Groves (1978) studied 21 normal patients who consumed coarse (83% particles 1500 microns) or fine (79% particles 710 microns) bran. Faecal wet weight was reduced significantly by 10% on fine bran. Smith et al (1981) have also compared the effects of fine milling of bran and have found the same effect on stool weight. The possibility that chemical structure might alter colonic responses has been investigated by Smith et al (1981). Canadian red spring wheat and French soft wheat were administered to subjects. Though expected responses to transit time, motility pressure index and faecal weight occurred, no differences in response were noted between the two sources of bran. Cummings et al (1978ii)

however studied cabbage, carrot, apple, bran and guar and found that pentose content correlated well with faecal output though the mechanism whereby this might be explained was not understood. Lignin content correlated with faecal bulk and it was felt this might contribute to water holding by resisting bacterial degradation of fibre.

A number of potential mechanisms have been proposed whereby D.F. may exert these effects. The known ability of D.F. to take up and hold water in its interstices has been used to explain its bulking action and effects on intestinal motility (Tainter and Buchanan 1954; Kirwan et al 1974). Thus Brodribb and Groves (1978) were able to show that reduction in particle size by milling reduced water holding capacity of bran by 47% and ability to increase stool weight by 10%. Nevertheless Stephen and Cummings (1979) examined the water-holding capacities and effects on stool weight of 17 different D.F. preparations including refined and unrefined cereal, vegetables and fruit, and isolates and found an inverse relationship between water-holding capacity and effect on faecal weight. Water-holding was high in highly charged D.F. molecules and in D.F. with a high uronic acid content. Thus pectin has a powerful water-holding effect but little activity on stool bulk or transit time. The authors questioned differences in analytical technique but concluded that the bacterial degradation of fibre was probably responsible for altering water-holding capacity in the colon. Bacterial degradation of D.F. and the resulting appearance of short chain fatty acids has been proposed as a means whereby faecal weight might be mediated by virtue of their osmotic effects. Williams and Olmstedt (1936ii) studied the patterns of V.F.A. production and laxation of 7 sources of D.F. and reported a rough parallel between stool weight and the presence of V.F.A. Hellendoorn (1978) has lent more recent support to this suggestion. However it is recognised that the principal V.F.A. in the faeces are readily absorbed by the colorectal mucosa of man (McNeil et al 1978; Ruppin et al 1980). Though Fernandez et al (1971) have correlated organic anion output with faecal weight in diarrhoeal

states it has been shown that V.F.A. output concentration reduces as diarrhoea worsens (Cummings et al 1973) and it is likely that associations between V.F.A. and stool weight are fortuitous (Cummings 1975).

If V.F.A. is not a prime mediator in control of faecal weight, then bacterial action is still of importance. The degradation of fibre in the large bowel by bacteria has been widely recognised (Southgate et al 1976; Keretz 1940; Werch et al 1942; Holloway et al 1978; Cummings 1981ii). It is only recently that the major contribution of bacterial mass to stool weight has been recognised (Stephen and Cummings 1980i). 18g D.F. as bran was 36% digested and 48% of the increase in faecal weight was accounted for by faecal water while 10% represented bacteria. Cabbage (18.3g.d^{-1}) however resulted in bacterial solids which represented 35% of the increase in stool weight. In all bacteria represented approximately 55% of the faecal weight produced on a control diet. Faecal bacterial mass were correlated significantly with intestinal transit time and stool weight. Cabbage was highly metabolised in this study (92%) and still resulted in a significant increase in stool weight. Pectin and guar gum, both completely metabolised during intestinal transit, cause little increase in stool weight (20-33%) and may decrease it. Presumably considerable bacterial proliferation occurs during cabbage digestion. The discrepancies in stool weight alterations between these highly degradable fibres may be due to retarded transit times for pectin and guar compared to acceleration with cabbage fibre allowing more water absorption and therefore a reduction in bulk. Comparisons of relatively small alterations in faecal weight must be carefully balanced however, in view of the very variable nature of stool output in healthy subjects (Wyman 1978). Cummings et al (1978ii) recorded a 58% variation in responses in individuals to D.F. supplements.

Faecal Biochemistry and dietary fibre.

D.F. results in many alterations in faecal biochemistry not all of which are consistent. Most studies have concerned cereal fibre.

Faecal nitrogen excretion is perhaps most consistently increased after consumption of D.F. Increases have been recorded after consumption of supplements of bran (Cummings et al 1979ii); Cummings et al 1976; Rheingold et al 1976; Southgate and Duruin 1970), pectin (Cummings et al 1979iii; Nyman and Asp 1982), and after guar or sugar beet fibre (Nyman and Asp 1982). Cummings et al (1979ii) have shown that this effect is independent of protein intake. Previously this increase in excretion has been attributed to increased loss from endogenous sources (McCance and Walsham 1948) or to the excretion of unavailable protein complexes (Van Soest and McQueen 1973). It has also been suggested that "unavailable carbohydrate" may shield dietary nitrogen from ingestion (Southgate and Durnin 1970), though studies in ileostomists demonstrate that increased nitrogen excretion does not occur after D.F. supplements (Sandberg et al 1981). Recent studies, though, indicate that increased faecal nitrogen excretion occurs as a result of colonic bacterial proliferation (Stephen and Cummings 1980i). Stephen and Cummings (1979) have shown that after cabbage consumption bacterial degradation of the fibre is almost complete and that 63% of the observed increase in faecal nitrogen is in the form of bacterial nitrogen while the increases in faecal nitrogen after bran have a contribution of only 34% from bacteria in keeping with its much lower digestibility.

Faecal fat excretion increases after the administration of bran (Walters et al 1975), Stasse-Wolthius et al 1980; Tarpila et al 1978; Southgate et al 1976), pectin (Kay and Truswell 1977; Cummings et al 1979iii) and after rolled oats (Judd and Truswell 1981). The mechanism for this increase is uncertain. Cummings (1978) has suggested that D.F. may shield dietary fat from digestion. Alternatively increases may be caused by the presence of fat in ingested

fibre (Wiggins quoted in Cummings 1978). Walker (1975) suggested that increases might be due to endogenous sources such as from exfoliated intestinal cells. Clearly the first two explanations would be suitable for relatively undigested fibre but would not explain the definite effect of highly metabolised polysaccharide such as pectin. Here it is possible that increases in faecal fat result from an increase in bacterial numbers, the fat being that present in the cell wall (Stephen and Cummings 1980). Intriguingly, pectin is known to reduce small intestinal mucosal cell turnover (Jacobs 1981) but also appears to result in microscopic mucosal damage (Cassidy et al 1980) which might relate to Walker's suggestions (Walker 1975) of increased endogenous losses. Thus the mechanism of alteration of faecal fat probably varies with source of D.F. and the effects on absorption and mediated through alterations in bacterial flora are probably most relevant.

Bile acid excretion has been widely studied in relation to a variety of sources of D.F. with no definite conclusion as to its effects. Dilution of faecal bile acid concentrations when bran is consumed is a relatively constant feature suggesting a water-holding effect (Tarpila et al 1978; Walters et al 1975; Eastwood 1973, Cummings et al 1976). However daily excretion of bile acids after bran has been demonstrated both to increase (Cummings et al 1976, Findlay et al 1974, Cummings et al 1979i) and to decrease (Tarpila et al 1978, Stasse-Wolthius et al 1980). Pectin ($15-36\text{g.d}^{-1}$) increases bile acid excretion (Cummings et al 1979iii, Kay and Truswell 1977) as does bengal gram (Mathur et al 1968), bagasse (Walters et al 1975), and rolled oats (Judd and Truswell 1981). A mixed diet containing cereal, vegetable and fruit fibre ($\text{C.}60\text{g.d}^{-1}$) had no significant effect on bile acid excretion (Raymond et al 1977).

The reasons for such wide variation in response in bile acid excretion after fibre remain unclear though certainly the wide variation in response of individual subjects to manipulations in fibre intake have a major impact on the results and interpretation of studies of bile acid excretion. (Bell et al 1981). It is widely known that bile acids will bind to many types of fibre in

vitro (Eastwood and Hamilton 1968, Kritchevsky and Story 1975) and to bacteria and other faecal solids (Heaton 1982). However colonic bacterial activity totally degrades pectin, a strong bile acid binder, and yet in modest (15g.d^{-1}) or high (36g.d^{-1}) dosage it increases faecal bile acid excretion. The explanation is unclear, though Pfeffer et al (1981) have demonstrated that the apparent bile acid binding of pectin is probably attributable to the presence of low molecular weight contaminant materials. After repeated centrifugation to remove diatomaceous earth and further purification using tubing dialysis they demonstrated no bile acid binding activity by pectin but considerable activity by the separated contaminants. Possibly bacterial proliferation and binding may play a role for bile acids malabsorbed by the small bowel due to pectin binding. If bacterial proliferation occurs after pectin consumption it does not alter metabolism of faecal sterols since the ratios of primary:secondary acid and neutral sterols remain constant (Kay and Truswell 1977, Ross and Leklem 1981). This is in contrast to the effects of cereal fibre which while generally ineffectual in altering output result in an apparent protection of primary sterols from bacterial attack and thus an increased primary:secondary ratio (Ulrich et al 1981).

Both pectin and guar cause significant rises in faecal neutral sterol excretion (Kay and Truswell 1977, Stasse-Wolthius et al 1980). Ross and Leklem (1981) found no alteration in neutral sterol excretion after pectin (15g.d^{-1}) added to a standard diet. These authors concluded that dietary differences were insufficient to explain the variance with previous results, but cholesterol intake (260mg.d^{-1}) was 39% less than in Kay and Truswell's study (425mg.d^{-1}). Bile acids were unaffected in the study though total faecal sterol excretion rose significantly suggesting that ingested dose of cholesterol may have been important. This appears to be a property largely attributable to the water soluble or gel forming polysaccharides since cereal bran, vegetable and fruit fibre have a varying effect, in general producing no significant alterations in output (Stasse-Wolthius et al 1980, Raymond et al 1977, Ulrich et al 1981). The

mechanism appears to be related to malabsorption of sterols during small bowel transit though this remains unproven in man. Raymond et al (1977) studied ^{14}C -cholesterol absorption by subjects who consumed diets low or high (60g plant cell wall material, 16g crude fibre $\cdot\text{d}^{-1}$) in D.F. and containing 1,000 mg cholesterol. ^{14}C -cholesterol absorption was insignificantly decreased (44% to 42.9%) by the addition of this large quantity of fibre which contained pectin in an unstated proportion. Results of other studies are more difficult to interpret since cholesterol intake was usually neither controlled nor specifically measured. In rats fed elemental diets for six weeks and to which were added fibre supplements, alfalfa, cellulose pectin, and cholestyramine significantly reduced ^3H -cholesterol absorption assessed by lymphatic outflow. Pectin (15%) had the least effect and cellulose (15%) the maximum, greater than cholestyramine though this was administered in much lower dosage (2%).

In addition to altering the excretion of complex molecules, changes may occur in the excretion of electrolytes and minerals in the faeces. Thus faecal sodium, potassium, phosphate, chloride, magnesium, iron, calcium and faecal energy may all increase after feeding D.F. (Cummings et al 1976, Pomare and Heaton 1973, Southgate et al 1976). Cummings (1978) has pointed to the generally similar increases seen in bile acid fat and nitrogen excretion after cereal fibres. These are small compared to the effects of, say, a specific bile binding agent such as cholestyramine, and may be no more than a non specific effect possibly related to the chemical composition of the D.F.

Some effects of D.F. on small intestinal absorption.

The administration of D.F. is known to affect the absorption of minerals from the small intestine. Most studies have concerned cereal fibre. Calcium, magnesium, zinc and phosphorus absorption were all reported to be reduced during short term administration of wheat bran to human subjects (McCance and Widdowson 1942i) (Rheingold et al 1976).

Links between calcium metabolism and fibre intake here come from studies of populations who routinely consume high quantities of D.F. A high incidence of osteomalacia in immigrant populations (Arneil 1975), relatively deprived of sunlight for cultural reasons, could be linked to a high intake of D.F in the form of food such as chaputtis. Biochemical manifestations of osteomalacia can be reversed by Vit D or by exposure to ultra-violet light whilst a high fibre diet was consumed (Pietrek et al 1976). A similar biochemical though not necessarily clinical improvement can be induced simply by a reduction in the intake of dietary fibre and phytate (Ford et al 1972). Thus children and adults with clinical and biochemical osteomalacia treated with a chaputty free diet for seven weeks had at the end of the study period calcium and alkaline phosphatase concentration indicative of healing. The precise mechanism of calcium imbalance varies between the source of fibre, and the phytate content of the diet and is related to calcium malabsorption. Hence McCance and Widdowson (1942ii) were able to demonstrate the induction of negative calcium balance in subjects who switched from a white, low phytate bread to high phytate brown bread. Furthermore removal of phytate from brown bread caused increased absorption of dietary calcium although to levels rather below those associated with white bread (McCance and Widdowson 1931iii). Though phytate will bind calcium strongly, D.F. itself will bind calcium (McConnell et al 1974; Janes et al 1978) in vitro and may therefore contribute to alterations seen in calcium imbalance. However extrapolation between in vivo performance of fibre in vitro to effects it demonstrates in vivo must be viewed with caution. Pectin, a powerful binder of cations, was associated with a calcium balance identical to that on a control diet in contrast to a marked negative balance induced after three weeks on a wholewheat supplemented diet (Cummings et al 1976, Cummings et al 1979 iii). Strong calcium binding by pectin might have reduced calcium absorption by the small bowel but subsequent fermentation by bacteria will result in liberation of calcium in the colon whence absorption may occur.

Alterations in calcium balance have generally been

observed in relatively short term, acute, experiments and these may not accurately reflect the effects of chronic administration of D.F. Rheingold et al (1976) studied mineral balance for 20 days in subjects who consumed wholemeal bread supplements. Though calcium, magnesium, zinc, and phosphorus imbalance was induced through increased faecal losses, the imbalances appeared to lessen with the duration of the diet. This is in agreement with findings in prisoners whose calcium intake was reduced by 50%. Though most adapted satisfactorily to the diet the time taken to regain calcium balance was considerable in some subjects (Malm 1958). Therefore short term effects of fibre do not necessarily persist in the long term, emphasising the importance of long term studies in judging potential detrimental or beneficial effects of D.F. Nevertheless it appears that phytate is, in a quantitative sense, more important to calcium absorption than is the ability of a fibre source to bind calcium.

Iron absorption is also affected by the consumption of D.F. though like calcium it is strongly bound to phytate (McCance et al 1943). Studies of the effect of wheat bran on iron balance are likely to have been influenced by a marked phytate effect. Nevertheless conflicting evidence of the effects of D.F. on iron absorption exist. Simpson et al (1981i) administered bran supplements (12g.d^{-1}) to subjects and demonstrated a reduction in absorption of ^{55}Fe or ^{59}Fe . This effect was still present after removing phytate and was most prominent in the water soluble phosphate rich fraction of the phytate free bran. Sanstead et al (1978) were unable to demonstrate any evidence of iron imbalance after administering untreated wheat or corn bran (26g.d^{-1}), though in larger doses (36g.d^{-1}) Jenkins et al (1975) found reductions in serum iron, M.C.H. and MCV levels after only three weeks. Sandberg et al (1982) administered bran (16g.d^{-1}) to ileostomists. Though zinc absorption was impaired iron absorption remained unchanged. Recent iron experiments in animals suggest that in the rat at least, phytate appears to be the major arbiter of iron absorption. Fairweather-Tait (1982) administered white, brown and wholemeal bread of constant phytate ($6.2\text{--}6.4\text{g.kg}^{-1}$) content to rats and

measured the uptake and excretion of Fe after 14 days. Despite finding reduced iron uptake from each bread diet compared to an isoferic control diet.

Zinc malabsorption has been linked with D.F. intake, reductions in zinc retention being found after consumption of corn or wheat bran (Sandstead et al 1978). Evidence from ileostomies suggest that this effect may be truly related to dietary fibre since zinc output in the ileal effluent did not correlate with phytate excretion (Sandberg et al 1982). Epidemiological evidence suggests that zinc balance may be adversely affected in women who consume a vegetarian diet (Freeland-Graves et al 1980).

Thus though D.F. may cause short term imbalances, evidence of satisfactory adaptation to high fibre diets exists. Epidemiological evidence suggests that long term effects of fibre are minimal - vegetarianism appears to result in no long term ill effects such as anaemia or osteomalacia. Any imbalances that occur therefore may only be clinically relevant in subjects already exposed to risk because of social customs, as a result of Vit D deficiency or due to chronic blood loss.

Eastwood and Kay (1979) have outlined mechanisms whereby D.F. may have potential to alter small intestinal absorption. Gel formation or water folding capacity may trap soluble elements in the entrapped water or may be held by physical or chemical means in the gel structure thus retarding diffusion to sites of absorption. Furthermore the highly charged external surface of the fibre molecule may act as a cationic binder and prevent mucosal absorption. Bile acids may also be bound and result directly in cholesterol malabsorption or indirectly by increasing cholesterol utilisation through a reduction in bile acid pool size. Absorption may also be affected by changes in small bowel transit time. Finally it has been suggested that alterations in small intestinal mucosal morphology or kinetics might have links with alterations in small bowel function.

Effects of D.F. on glucose homeostasis.

Many forms of dietary fibre have been shown to alter glucose homeostasis in man. Guar gum and pectin when mixed with standard test meals result in reductions in the post prandial rises in plasma glucose and insulin in normal subjects (Jenkins et al 1977i). Wheat bran, methyl cellulose, cholestyramine and gum tragacanth will also reduce plasma glucose responses following a standard oral glucose tolerance test (Jenkins 1978 et al). This effect is not caused by malabsorption of ingested glucose since cramps, the production of flatus, or the elevation of breath hydrogen excretion do not occur (Jenkins et al 1976i; 1977i). Two mechanisms of action of D.F. are thought to be relevant. 1. A delay in gastric emptying. 2. A slowing in the rate of absorption of glucose from the lumen. Both mechanisms might permit complete absorption of a glucose load though at a slower rate. Both pectin and gum guar have been shown to increase gastric emptying time (Holt et al 1979, Schwartz et al 1982). Furthermore the rate of absorption of paracetamol from the small bowel was reduced after guar and pectin (Holt et al 1979).

From studies on a patient who had previously undergone a total gastrectomy and in whom responses to an oral glucose load were unchanged by the addition of guar and pectin it was concluded that their major effects were mediated through alterations in gastric emptying. Animal experiments do not entirely support this however, and Rainbird and Low (1982) have shown that pectin has no effect on the rate of gastric emptying in the pig, nor do bran, Lejguar gum, pectin and high viscosity carboxymethyl cellulose. Guar gum will significantly retard the diffusion from dialysis bags (Taylor et al 1980). Blackburn and Johnstone (1982) have shown that gum guar will reduce absorption of glucose from perfused segments of cannulated jejunum at doses of 6g.l^{-1} , and it seems likely that such inhibition of absorption is a most important property of the gel forming polysaccharides. In vivo it is likely that the increased barrier to diffusion caused by the increased viscosity of the unstirred layer causes a slowing of

glucose absorption (Jenkins et al 1978). Such slowing of absorption is accompanied by an increase in small intestinal transit time caused by viscous substances such as guar, tragacanth and pectin and this allows absorption to be completed before the small intestinal content reaches the caecum.

This euglycaemic property of D.F. is, as are many fibre related phenomena, not shared by all sources of D.F. Jenkins et al 1978 have pointed to the importance of viscosity of the fibre and have found a linear relationship between this factor and the reduction of the peak rise in blood glucose. Gums guar and tragacanth are most effective at reducing the post prandial glucose rise though the effect of gum guar can be neutralised by employing gum of low viscosity. The mode of ingestion of D.F. may also influence its effect on glucose absorption. Williams et al (1980) has shown that intimate mixing with food is required for fibre to exert its effect. Furthermore Haber et al (1977) have demonstrated by feeding whole apple, apple puree, or apple juice that increased refinement of a fibre source will result in less inhibitory effects on post prandial glucose rise.

The effects of fibre on glucose tolerance in normal subjects has been translated into therapeutic practice in the treatment of diabetes. There has been widespread advocacy of the use of fibre supplements in both insulin dependent and independent diabetes. The control of insulin dependent diabetes has been improved by the administration of diets high in unprocessed cereal and vegetable fibre ($60-90\text{g.d}^{-1}$) (Simpson et al 1981ii, Anderson and Ward 1978). Fasting glucose concentrations were reduced and in those patients on supplemental insulin doses of less than 30 units d^{-1} , these were discontinued. Guar and pectin (16 and 10g.d^{-1} respectively) can significantly reduce glucose and insulin concentrations up to 120 minutes after a standard oral glucose tolerance test (Jenkins et al 1976) and Konjac mannan, an unabsorbed glucomannan has a similar effect on fasting glucose levels, and post prandial insulin and glucose concentrations.

Like guar gum it also possesses hypocholesterolaemic properties (Doi et al 1979).

Insulin dependent diabetes may also be favourably affected by the administration of fibre. Jenkins et al (1976) found a flattening of oral glucose tolerance curves during the administration of a mixture of guar and pectin to three insulin dependent diabetics. Guar gum (25g.d^{-1}) alone has been found to reduce urinary glucose excretion and insulin requirements although the effect of the latter was only temporary (Jenkins et al 1977ii) and after two weeks treatment insulin doses were the same as before treatment began. Monnier et al (1981) demonstrated that diets high in vegetable fibre will also reduce post prandial rises in glucose, and that this effect seemed more prominent in labile diabetics.

Fibre consumption may however be only a part of the control of glucose homeostasis. Jenkins et al (1980i) studied 19 insulin dependent diabetics who consumed gum guar in the form of crispbread and a diet high or low in carbohydrate in the form of starchy carbohydrate not monomers. The high carbohydrate diet resulted in a 31% greater reduction in urinary glucose than the low carbohydrate diet though there appeared to be no linearity in relationship between glucose output and carbohydrate intake.

Despite much favourable comment however the uncritical advocacy of fibre in diabetes has been brought into question (Anon. 1981). Most suitable sources of fibre are unpalatable and since they are often of high viscosity difficult to incorporate into foods. In addition, side effects of non absorbable polysaccharides such as increased stool bulk, frequent bowel motions, ready satiety, and increased flatus production all combine to lower patient compliance. Nevertheless the maintenance of a diet rich in dietary fibre will usually be advised in the management of diabetes (Baird 1981).

A rather smaller therapeutic role for viscous polysaccha-

rides in controlling post prandial glucose alterations has been established in the treatment of the so-called dumping syndrome consequent upon gastric resection or vagotomy. Pectin has been shown to raise plasma glucose levels in patients in whom hypoglycaemia occurs after eating (Jenkins et al 1977iii, Jenkins et al 1980ii). Leeds et al (1978) confirmed these findings and also demonstrated a reduction in the post prandial fall in plasma volume seen in 6 patients with dumping syndrome. Jenkins et al (1980ii) have, in addition, demonstrated a "normalisation" of post prandial Endocrine responses by reductions in enteroglucagon and G.I.P. levels. They suggested that alterations in these gastro intestinal hormones were the primary mediators in the reduction of post prandial hypoglycaemia. In each of these three studies pectin was shown, in addition to its hormonal and biochemical effects to effect a clinical improvement in the majority of patients studied.

Dietary fibre and serum lipids.

Possible connections between the long term intake of dietary fibre and the subsequent incidence of atherosclerosis have promoted considerable study into the effects of D.F. on lipid and in particular cholesterol concentrations in the blood. As in other situations, D.F. has varying effects on serum lipids. Truswell (1977) reviewed 10 studies of the effect of bran supplements on serum lipid levels and found that in only one was a significant reduction in serum cholesterol achieved (Persson et al 1975) and in one a reduction in serum triglycerides (Heaton and Pomare 1974). Other studies have recorded increases in serum cholesterol after bran treatment (Jenkins et al 1975, Stasse-Wolthius et al 1980, Van Dokkum et al 1982). Though very high doses of cellulose caused a reduction in serum cholesterol in children (Shurpelaker et al 1971) hypocholesterolaemic responses to D.F. have been generally confined to its water soluble or gel forming polysaccharide components. Pectin is a consistently effective agent in normal subjects (Keys et al 1961, Palmer and Dixon 1966, Jenkins et al 1975, Durrington et al 1976, Kay and

Truswell 1977, Stasse-Wolthius et al 1980) and in those with documented hypercholesterolaemia (Balabanski et al 1982). Gum guar is also an effective hypocholesterolaemic agent (Jenkins et al 1975) (Kahn et al 1981) and similar responses have been found for rolled oats (de Groot et al 1963) which contains high concentrations of water soluble polysaccharide, leguminous vegetables (Grande et al 1965, Hellendoorn 1976), carrot fibre (Robertson et al 1979) and after bengal gram (Mathur et al 1968) serum phospholipids and triglycerides have been studied less and though Heaton and Pomare (1974) found reductions after consumption of bran most studies have failed to demonstrate any effect for bran (Eastwood 1969, Jenkins et al 1975, Walters et al 1975), oats (Kirby et al 1981), bagasse (Walters et al 1975), gum guar (Kahn et al 1981) or for carrot fibre (Robertson et al 1979).

The efficacy of water soluble polysaccharides in altering serum lipids in normal and hyperlipidaemic patients has clinical applications, but their use is often limited by palatability and the relative large quantities that must be consumed to achieve a useful effect. Thus Durrington et al (1976) have calculated that in order to consume pectin at a therapeutic dose ($c.12g.d^{-1}$) would require a subject to ingest 1.2 kg of apples per day.

The mode of action of D.F. in lowering serum cholesterol levels is uncertain though a number of possibilities exist. Where faecal bile acid excretion is increased, for instance after pectin ingestion (Kay and Truswell 1977; Stasse-Wolthius et al 1980), increased bile acid loss may deplete the bile acid pool and result in cholesterol malabsorption. Furthermore replenishment of bile acids will result in increased use of endogenous cholesterol for their synthesis. D.F. may also bind cholesterol and inhibit absorption by the small intestine. Increased neutral sterol concentrations after pectin consumption would support such a proposal (Kay and Truswell 1977, Stasse-Wolthius 1980). Thirdly by their known inhibitory effect on glucose absorption there may be a reduction on the glucose load on the liver which with reductions in insulin levels may result in a lowering of the stimulus to

cholesterol production (Kahn et al 1981). It has been suggested that altered absorption of V.F.A. may result in alterations in cholesterol synthesis (Anderson and Chen 1979).

Animal studies have produced conflicting findings with regard to the ability of fibres to reduce serum cholesterol levels. Reductions in serum cholesterol have been achieved with pectin, rolled oats, and gums guar and arabic in rats (Kiriyaama et al 1969, Kelley and Tsai 1978, de Groot et al 1963). However Tsai et al (1976) have pointed to the inconsistent results obtained with rats fed a number of fibre sources and to the important effects that dietary interactions may have on cholesterol levels. Furthermore he emphasised the relative resistance of rats to changes in cholesterol levels in serum after dietary alterations. Hegstedt (1977) has indicated that the rat is a poor model for studies on dietary fat and cholesterol. Nevertheless studies in rats do suggest that the hypocholesterolaemic effect of pectin and gum arabic is due to impaired absorption (Kiriyaama et al 1969). Furthermore oral gum arabic has been shown to reduce synthesis of ^{14}C sterols after intravenous injection of ^{14}C glucose though unlike pectin it does not alter cholesterol turnover (Kelley and Tsai 1978). No evidence exists as to the ability of G.A. to reduce serum cholesterol in humans.

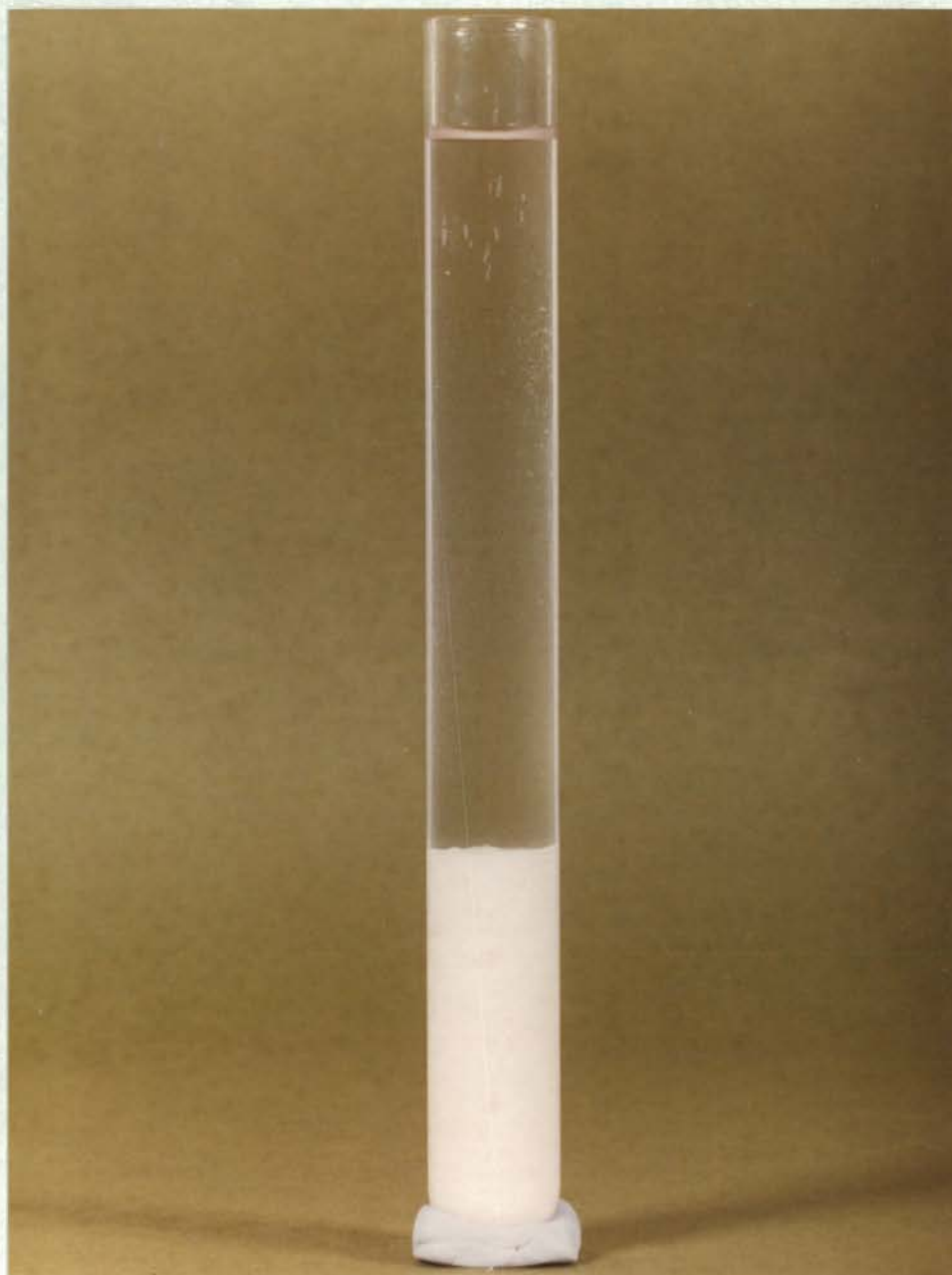


Fig.1: The typical appearance of a flocculent white precipitate of gum arabic after precipitation by acidified ethanol from an aqueous solution.

Methods

Animal Experiments

Animals

Liverpool-Hooded rats bred in the University of Edinburgh, Usher Institute animal facility were used for the long term (2 year) study of gum arabic ingestion. 2 month old c.120 gram male and female rats were chosen at commencement of the study.

Albino Wistar rats either purchased from Messrs. Bantin & Kingman, Hull or bred in the University of Edinburgh, Western General Hospital animal facility were used for all other animal experiments. In general 200 - 300 gram rats were chosen. Occasionally rats were selected on the basis of maturation which was considered to be reached at 12 weeks.

Housing

Animals were housed initially at the registered animal house located at the Usher Institute, University of Edinburgh, and subsequently at the registered animal unit, Western General Hospital, Edinburgh. Housing conditions were the same at both units. Animals were housed at a maximum of three per cage; this was reduced to two or one per cage when necessary for experimental or hygienic reasons. The lighting cycle used was 12 hours on and 12 hours off (7.30 p.m. to 7.30 a.m.). There were 10 - 11 filtered air changes per hour. Ambient temperature was maintained between 20 and 21°C.

Diets

Three forms of rat diet were employed.

(i) Standard diet:

The standard laboratory rat diet used prior to feeding experiments was an autoclaved Spratts (Spillers) small animal diet.

(ii) Reconstituted pellet diet: (OBD; OBD + GA).

This diet was devised to allow the incorporation of gum arabic (OBD + GA) into pellet form similar to that of the standard diet. The diet was based on pellets of oxid breeders diet (OBD) (Oxoid Limited, Basingstoke). A breeders diet was chosen for its high protein content in order to minimise the nutritional consequences of incorporation of gum arabic into the diet. The method of preparation of the reconstituted diet was as follows:

Oxoid breeding diet pellets were ground to a powder, and powdered gum arabic incorporated at concentrations of 2.5%, 5.0%, 10.0% and 20.0% by weight. Water was added to make a thick paste and this was extruded into 0.5 inch diameter rolls which were dried on stainless steel trays overnight at 70 - 75°C. The effects of the baking process and the inclusion of gum in the pellet diet on protein content have been examined in other experiments (McNab et al - unpublished observations) (Table 1). Alterations in proportions of individual amino acids are noted in Table 2. The "ideal formulation" of amino acids is based on an ideal minimum protein intake of 200 g.kg⁻¹ (Coates et al 1969).

(iii) Elemental diet: (Elem D; Elem D + GA).

A low residue, nutritionally complete diet into which gum arabic could be incorporated to a concentration of 12.9% dry weight was devised. The details of its manufacture are as follows:

Gelatin (30g) were dissolved in distilled water (600ml). Flexical powder (Mead Johnson Laboratories, Slough, 240g) were dissolved in this solution. The mixture was poured into 30 moulds and allowed to set. When gum arabic was incorporated, the gum arabic (40g) was initially dissolved in distilled water (600ml) and the two steps described previously then carried out.

Diets were fed to the animals ad lib or in measured amounts when necessary. Scattering was not a significant problem with the pellet diet but scattering occurred frequently with the elemental diet and this made estimations of the weight of diet consumed less accurate.

The gum arabic used in the study was supplied by Messrs. Rowntree Mackintosh Ltd., York. Details of its purification are as follows:

Commercial gum arabic of appropriate foodstuffs quality, as used routinely in confectionary manufacture was dissolved in hot water and the resulting gum liquor treated by industrial filtration and centrifugation. This process removes particulate matter such as sand and bark. Gum arabic was recovered from the liquor as a pure white powder by spray drying. This gum arabic complied in all respects with the specifications listed in the British Pharmacopoeia (1980).

Energy and fibre contents of the pellet and elemental diets are noted in Table 3.

Sample collection methods:

(i) Urine:

Urine was collected from animals housed in metabolic cages (North Kent Plastic Cages Ltd.) which allowed separation of urine and faeces passed. Collections were usually conducted over a period of 16 - 24 hours.

(2) Faeces:

Faeces were collected from animals housed in broad spaced, grided cages to minimise the possibility of coprophagia. Collections were carried out over 24 hour periods except in animals on an elemental diet, for which 3 - 4 day collections were employed, each 24-hour aliquot of passed faeces being collected separately. After collection faecal samples were placed immediately in a refrigerator (-20°C).

(3) Blood:

Venous blood samples were obtained from the retro-orbital plexus using plain or silanized glass Pasteur pipettes. Animals were lightly anaesthetised with diethyl ether during this procedure. Blood was also obtained by cardiac puncture in certain instances.

Aortic blood was collected by abdominal aortic cannulation below the origin of the mesenteric artery using a P.V.C. or silicone elastomer catheter 1 - 2mm in diameter.

Intestinal content:

The contents of the stomach, small bowel, caecum and left

colon were collected from animals sacrificed by a combination of diethyl ether anaesthesia and cervical dislocation prior to abdominal evisceration.

Stomach, small bowel, caecum and distal colon were immediately isolated and their content evacuated into plastic containers. Caecum and stomach were opened to ensure complete collections. After harvesting the intestinal contents were deep frozen immediately (-20°C) prior to freeze drying. Fresh samples were used in certain instances.

Intestinal content was always collected between 0900 and 1200 hrs. to ensure maximal and constant intestinal filling after overnight feeding.

Freeze-drying.

Samples for freeze-drying were deep frozen (-20°C) for at least 24 hours, and then placed in universal containers in a Pirani 10 freeze-drier (Edwards Laboratories) operating at -40°C and 10^{-1} atmospheres. Samples were processed for 48 hours, then stored in air tight containers at room temperature.

Excreted hydrogen/methane

Single animals were placed in an air tight perspex box (dimensions 30 x 20 x 20 cm; volume 12 litres) for 15 minutes. Samples (50ml) of headspace gas were taken for analysis at the end of that time. Background room air samples were taken before and after each set of animal measurements.

Chemical Methods

Gum arabic identification

(a) Ethanolic precipitation:

Gum arabic in aqueous solution (1 volume) was precipitated out by addition to acidified ethanol (4 volumes). Where precipitation from intestinal content was required water was added (1:1 v/v)

to dilute and the suspension then spun at 9,100G for 20 minutes to remove suspended mucoid or proteinaceous material to allow minimum precipitation of these substances after addition to ethanol. The typical white flocculent appearance of the gum arabic precipitate is seen in Figure 1 Chapter 1.

(b) G.A. hydrolysis

Dried samples were subjected to an acid hydrolysis known to convert gum arabic into its constituent sugars - arabinose, galactose, rhamnose and glucuronic acid. The details are:

The sample (5 - 50mgs) was hydrolysed with IN-Sulphuric acid for 7.5 hours on a water bath (100°C). The hydrolysates were then neutralised with barium carbonate, filtered, deionised with Amberlite IR-120(4) resin overnight and concentrated on a rotary evaporator at 37°C.

(c) Chromatographic techniques:

Thin layer and paper chromatography were used to separate the sugars present in the hydrolysate.

(i) Thin layer chromatography (TLC)

The method used was that described by Anderson & Stoddart (1966). Commercial Silica Gel coated plates impregnated with a 0.1N boric acid solution were used (Eastman Chromagram Sheet 6061, Distillation Products Industries).

A near vertical ascending technique was used in a small tank lined with filter paper to assist vapour phase equilibration.

The solvent used was butan-1-ol/acetone/water (ratio 4 : 5 : 1). Separation time was approximately 3 hours. After drying, the chromatograms were sprayed with a saturated solution of ethanolic analine oxalate and developed at 140°C for 2 - 3 minutes. Samples (2 - 4µlitres) were applied and the separated sugars were identified by comparison with sugar standards run on the same chromatograms.

(ii) Paper chromatography

Paper chromatography of sugars was carried out on Whatman No. 1 paper. The solvent system used was benzene, butan-1-ol, pyridine, water (1 : 5 : 3 : 3, V/V). A vertical descending technique was used and the sides of the chromatography tank lined with filter paper to assist vapour phase equilibrium. Separation time was about 24 hours. Chromatograms were developed by spraying



with a saturated solution of analine oxalate in ethanol/water (1 : 1 V/V) followed by heating at 105°C for about 10 minutes.

Short chain volatile fatty acids (V.F.A.)

The method used was a modification of that described by Spiller et al., (1980). V.F.A. concentrations were estimated on freeze-dried samples of intestinal content or faeces.

The sample (c. 0.1g) was accurately weighed; ortho-phosphoric acid (100µl) distilled water (0.8ml) and the internal standard, methyl valeric acid (50µl) were added to the sample and thoroughly mixed. V.F.A.'s were extracted into redistilled diethyl ether (3ml). The extraction was repeated twice on the same sample and the three extracts were combined. V.F.A. separation was achieved by gas liquid chromatography (G.L.C.). Samples (2 - 3µlitre) were injected into an SP2250 filled glass column, using nitrogen as a carrier gas at a flow rate of 60ml/minute. Temperature programming increased column temperature from 80 - 150°C at 16°C.min⁻¹.

Sample peaks were recorded on a Honeywell Electronic 194 recorder. Individual V.F.A. peaks were compared with the internal standard peak to obtain peak height ratios. V.F.A. concentrations were derived from standard curves constructed from serial dilutions of each V.F.A. each dilution containing a constant quantity of an internal standard.

The V.F.A.'s assayed were acetic, propionic, isobutyric, butyric, isovaleric and valeric.

Concentrations were expressed as mg.V.F.A.g⁻¹ freeze dried faeces.

Hydrogen and methane estimation:

The method used was that described by Tadesse et al., (1979). Gas samples were analysed on a Pye Series 104 gas chromatograph using a Katharometer detector. Samples (25ml) were

injected on to a glass column packed with molecular sieve size 5A, 60/85 mesh. Nitrogen was used as a carrier gas at a flow rate of 65ml.min⁻¹.

Sample peak heights were compared with standard concentration peaks and results expressed in ml.kg⁻¹.h⁻¹.

Laboratory analysis:

Urinalysis:

Urinary pH was measured and the presence of ketones, blood, glucose, protein, bilirubin and urobilinogen detected in a semi quantitative fashion using Ames Multistix. Reducing sugars were detected using Fehling's test.

Haematology:

Haemoglobin, haematocrit, white blood cells, mean corpuscular haemoglobin concentration, mean cell volume and red blood cell count, were estimated using a Coulter S analyser, by courtesy of Dr. N.C. Allan, Department of Haematology, Western General Hospital, Edinburgh.

Biochemistry:

Serum, albumin, total protein, urea, sodium, potassium, total CO₂, alanine amino transferase, alkaline phosphatase, calcium, phosphate, creatinine, uric acid and aspartate amino transferase, were analysed using sequential multiple analysis (SMAC 1 - Technicon Instruments Co., Basingstoke, England) by courtesy of Dr. Percy-Robb, Royal Infirmary of Edinburgh. Trace element estimations of gum arabic samples were estimated by Dr. Allan Shenkin, Department of Clinical Pathology, Royal Infirmary, Glasgow.

Histopathological Techniques

Small intestinal morphology

The technique used was based on that described by Clarke (1970i). Animals fed standard and gum-containing diets were sacrificed by ether anaesthesia and cervical dislocation. Samples of mid jejunum approximately 1cm long were immediately removed and placed in Clarke's solution (75% absolute alcohol, 25% glacial acetic acid) for 24 hours and thereafter placed into 75% alcohol until examined. Specimens were stained by Fielgen's method. Samples were washed in reducing quantities of alcohol and distilled water, and then hydrolysed in 1M HCl for 20 minutes at 60°C. After washing in water they were stained with periodic acid Schiff reagent for 30 minutes, and then transferred to water until processed. Using a dissecting microscope, the serosal, muscular, and submucosal intestinal layer were removed leaving the mucosa from which were cut thin slices as near to one villus and crypt thick as possible. Satisfactory slices were then examined under a Leitz Dialux 20 E.B. microscope and crypt depth and villus height measured using a calibrated micrometer eyepiece. 10 values for each parameter were measured and a mean obtained.

Ileal cell turnover studies:

Rats fed gum supplemented or regular diets were injected with Vincristine sulphate ("oncovin") (1mg.kg^{-1} . body weight⁻¹) by the intraperitoneal route. Rats were then sacrificed by ether anaesthesia and cervical dislocation at known time intervals after vincristine injection (30, 60 and 90 minutes). Mid jejunal samples approximately 1cm long were taken and immediately placed in Clarke's solution for 24 hours and thereafter transferred to 75% alcohol for storage until examination.

Isolated jejunal mucosal slices were prepared and examined as above. Mucosal crypts were then isolated by mucosal disruption using gentle pressure on a cover slip. Cells arrested in metaphase were then identified and the number in each crypt recorded. Ten

values were obtained per specimen and an average calculated.

From the mean values, obtained for each elapsed time interval from injection, a linear regression line was calculated and from this the cell production rate derived and expressed in cells per crypt per hour. Cell production rates on different dietary regimens were compared by the method of analysis of covariance.

Histopathology

Fixation:

Specimens were removed and placed immediately in normal saline solution for fixation and storage.

Staining:

Blocks were cut from fixed specimens and embedded in paraffin wax. Sections were cut and stained with haematoxylin and eosin stain, prior to examination under a light microscope.

Surgical procedures

Caecal excision

Rats were fasted on the night prior to surgery. An inhalational anaesthetic of air/ether was used. After shaving and skin preparation a mid line incision was made and the caecum exteriorised. The vascular supply was controlled, the caecum excised and an ileocolic anastomosis made end to end using interrupted 5.0 prolene. The laparotomy incision was closed in a mass suture of continuous 3-0 prolene with disposable clips to the skin. Rats were allowed fluids only for three days after the operation and were then gradually weaned on to a semi solid and finally a normal diet.

Statistical methods

The students t test or Wilcoxon ranked sum test was used

for paired or unpaired data. The least significant difference multiple comparison test described by Steel and Torrie (1960) was also employed where appropriate.

TABLE 1

Protein content of unprocessed, baked O.B.D. and baked O.B.D. + 20% G.A.

	<u>Protein Content</u> g.kg ⁻¹
Unprocessed Oxoid Breeder's Diet	227
O.B.D. after baking (85°C)	217
O.B.D. + 20% G.A. after baking (85°C)	188

TABLE 2

Amino acid proportions (% of total amino acid content) of unprocessed oxoid, baked O.B.D. and baked O.B.D. + 20% G.A. (tryptophan, methionine, and cysteine were not estimated).

	ideal pattern	unprocessed oxoid diet	O.B.D. baked 85°C	O.B.D. + 20% G.A. baked 85°C
arg	5.0	7.5	6.9	6.4
his	2.5	2.7	2.7	2.6
lys	6.0	5.8	4.7	4.6
tyr	4.0	4.2	4.2	3.7
try	1.5	-	-	-
phe	5.0	4.0	5.3	4.0
met +				
cys	4.5	-	-	-
thr	4.0	4.1	4.2	4.4
leu	8.0	8.6	8.2	8.4
Ile	5.0	4.7	4.6	4.4
val	5.5	5.4	5.4	5.0

TABLE 3

Energy and fibre contents of O.B.D. and Elem-D diets.

	<u>O.B.D.</u>	<u>ELEM-D</u> [*]
ENERGY	12.0 mj.kg ⁻¹	18.5 mj.kg ⁻¹
N.C.P.	10.8%	-
Cellulose	4.4%	-
Lignin	3.8%	-
Total D.F. (excluding lignin)	15.2%	

* Refers to quantities in unconstituted Flexical powder.

Gum arabic (G.A.)

The gum arabic used in the human studies was supplied by Rowntree Mackintosh, York. A 20% solution was used. In certain circumstances 7.5% anhydrous dextrose was added to the G.A. solution to add flavour. The gum purification process was identical to that described in the animal experiments section.

Sample collection

Faeces

Faeces were collected in purpose made plastic slings and frozen (-20°C) as soon after collection as possible. In general 5 day collections were made. The time and date of passage of each stool was recorded. After collection and freezing individual stools were weighed and X-rayed. All stools were then pooled, allowed to thaw, diluted in a known volume of water, mixed and homogenised. An aliquot of known volume was retained, refrozen and freeze dried under the conditions described in the animal section. Freeze dried faeces were stored at room temperature until use.

Hydrogen and methane

Exhaled methane and hydrogen concentrations were estimated using the method previously described from samples (50ml.) of end alveolar exhaled gas using a modified Haldane Priestley apparatus. Samples were taken at intervals over 4-6 hours.

Dietary estimations

Average daily intake of dietary constituents was estimated by analysis of diet diaries kept by subjects during periods of study, supplemented by a 24 hour dietary recall interview with a dietician. This analysis was made using a standard computer package in the Department of Dietetics, Western General Hospital, Edinburgh.

Intestinal Transit Time (I.T.T.)

I.T.T. was estimated using radio opaque plastic pellets as described by Hinton et al (1969). Each subject swallowed 40 pellets at a predetermined time. All stools passed over a five day period were collected, timed, and then X-rayed after freezing. I.T.T. was taken as the time taken for the 32nd pellet (80%) to appear in the stool.

Haematological estimations

The following estimations were carried out using automated Coulter or manual methods: white blood count, haemoglobin, haematocrit, red cell count, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, reticulocyte count, platelet count, blood film, erythrocyte sedimentation rate.

Biochemical estimations (Blood)

Heparinised plasma samples were analysed for the following parameters using the SMAC 1 system previously described: plasma proteins, albumin, SGPT, ALT, alkaline phosphatase, sodium, potassium, chloride, CO₂ content, urea, bilirubin, calcium, phosphate.

High density lipoprotein and total cholesterol

Samples of serum from fasting subjects was used. Low density lipoproteins were selectively precipitated with magnesium phosphotungstate and cholesterol in the soluble HDL fraction was estimated using the enzymatic method described by Allain et al (1974).

Glucose

Plasma glucose was estimated using an automated glucose oxidase method. Blood glucose was estimated from this value and the patient's haematocrit.

Insulin

Plasma insulin levels were estimated by a radio immuno-assay technique using single antibody and charcoal separation.

Tryglycerides

Samples of serum from fasting subjects were analysed using a Boehringer Mannheim Triglycerride kit (Egstein 1966).

Phospholipid

Samples of serum from fasting subjects were analysed using a method based on Boehringer's modification of the method of Zilver-smit (1950).

Biochemical analyses (Faeces)

Faecal neutral sterols

The following neutral sterols were measured: Coprosterol, epicoprosterol, cholesterol, campesterol, B sitosterol.

The method employed was that of Miettinen et al (1965). Internal standard (5B cholestane 1mg.ml^{-1}) (1ml) was dried down in a glass tube to which was added approximately dried faeces (1mg) accurately weighed, and 1 N NaOH in 90% ethanol (10ml). Tubes were then boiled at 85°C for 1 hour and the suspensions then filtered. An aliquot (2ml) was taken from each tube and neutral sterols extracted into petroleum spirit (5 mls) at $60-80^{\circ}\text{C}$ (x3). The petroleum spirit was then washed and filtered after addition of sodium sulphate. The filtrate was taken to dryness and sylon HTP (8 drops) added and left for 30 minutes.

0.1 μl aliquots were injected on to a 3% OV 17 filled GLC column. Concentrations were calculated by deriving neutral sterol peak/internal standard peak ratios and extrapolated from standard neutral sterol/internal standard curves.

Faecal bile acids

The following bile acids were measured: Lithocholic, Chenodeoxycholic, Deoxycholic, and cholic acid. Bile acids were measured in their methyl ketone forms. Nordeoxycholic acid was used as an internal standard.

Bile acids were extracted from samples of freeze dried faeces into toluene with an internal standard (23-nordeoxycholic acid) by boiling in acetic acid. Bile acids were then hydrolysed in KOH and neutral sterols extracted into petroleum ether. Bile acids were extracted into diethyl ether. Methylation of bile acids was achieved by addition of HCl and 2.2 dimethoxypropane, followed by oxidation using acidified chromic oxide. Bile acids were then extracted into diethyl ether.

The bile acids were separated in their methyl ketone form by GLC using a 3% OV 17 packed column at 270°C using nitrogen as a carrier gas. Bile and peak/internal standard peak ratios were calculated and concentrations calculated by comparison with standard bile acid/internal standard peak ratio curves.

Faecal fat

A petroleum ether extract was taken to dryness and faecal fat measured by titration with alkali.

Volatile fatty acids

The method used was that described in the animal methods section.

During the conduct of the work for this thesis the laboratory involved changed from expressing quantities of V.F.A. in mg to mmol. Thus human results are expressed in mmol. V.F.A. and mg. V.F.A. to allow comparison to be made.

Statistics

Paired samples were compared using the Wilcoxon rank sum test. Correlation coefficients were assessed for significance assuming a normal population distribution judged by graphs.

Chapter 3 (i)

Effects on rats of chronic ingestion of gum arabic.

This study was initiated to ascertain the effects of chronic administration of gum arabic to rats. Liverpool-Hooded rats were used and the period of study chosen was 2 years; the rats were 8 weeks old at the start of the study.

Animals were fed a reconstituted oxoid diet as described in Chapter 2 pp 47-49, processed gum arabic being incorporated at 0% (control) 2.5%, 5.0%, 10% and 20% dry weight. 5 male and 5 female animals were used in each of the 5 treatment groups. Food and water was available ad libitum, and housing conditions were as described in Chapter 2.

At the end of the study all animals were anaesthetised with diethyl ether by inhalation and blood was obtained from the retro-orbital plexus for biochemical and haematological estimation. Animals were killed by cervical dislocation. Full macroscopic post mortems were carried out; specimens were taken for histological analysis where abnormalities were detected.

Results

(a) Animal weights

Animal weights at sacrifice are shown in Table 1. Though there was some loss in final carcass weights in both groups (which was most marked in the females) statistically significant decreases in weight between the control and test animals were only observed in the group fed with 20% gum arabic (females $p < 0.05$, males $p < 0.001$).

(b) Urinalysis

Dipstick examinations (Multistix, Ames Laboratories) showed no differences between control and treatment animals at all dose levels. Proteinuria ($100\text{mg}\cdot\text{ml}^{-1}$) was found in all animals.

(c) Haematology

The results of full blood counts carried out on retro-orbital plexus blood removed prior to animal sacrifice are shown in Table

No significant differences were detected between any of the treatment groups and their controls at two years, though in some groups the figures were small for statistical purposes.

(d) Biochemistry

Serum obtained by retro-orbital plexus puncture was subjected to SMAC analysis. The findings are presented in Tables IIIA and IIIB.

Haemolysis during collection was a problem with some samples and caution must be used in particular when interpreting AST, ALT and potassium concentrations.

In Table IIIB, male and female figures for each male and female group have been added together to increase the group sizes for statistical purposes. It can be seen that serum sodium concentration was slightly but significantly higher in the 10 and 20% groups while serum urea was significantly lower in the 10% group. ALT concentrations were significantly reduced in the 5% dose group. Otherwise no significant variations from control values were discerned.

Pathology

Animals were killed by ether anaesthesia and cervical dislocation between 0900 and 1200 hours after ad lib overnight feeding to ensure constant and maximal intestinal filling.

At autopsy the caecum was immediately removed and weighed complete with its contents. A full post mortem was then carried out on each animal, though no intracranial examination was made. Any macroscopic abnormalities were biopsied and submitted to paraffin section histology.

Findings

(a) A constant finding in all male and female treatment groups was caecal enlargement. Table 4 demonstrates the increasing relative

weight of the caecum in female rats with increasing dose of gum arabic; a maximum found in the 10% diet ($r = 0.5$ $p < 0.005$). Similar findings were noted in male animals but collection difficulties resulted in insufficient numbers for analysis.

(b) Pathological abnormalities were few and are enumerated in Table V. No differences in incidence of pathological complications were found between the various treatment groups.

Five animals died prematurely, equal numbers in control and treated groups being involved. At autopsy only one of these animals had abnormality which might have caused the death. This was a diaphragmatic hernia in a male animal in which approximately one third of the small intestine lay in the left hemi thorax. There was no evidence of intestinal obstruction however, and although the lung was compressed no other pathology was visible.

SUMMARY

Male and female adult rats were fed varying doses of processed gum arabic to ascertain whether any long term ill effects result from the chronic ingestion of this complex polysaccharide.

Animals fed a diet containing 20% gum arabic had significantly lower body weights at two years when compared with their controls, with a trend towards lower final body weights with increasing doses of gum arabic. These decreased body weights are believed to be reflections of nutritional inadequacies in the diet consequent upon gum arabic incorporation rather than a direct toxic effect of gum arabic as will be demonstrated in

No significant differences between control and treated animals are apparent in the haematological indices. However, several biochemical indices show variations from control values, though no dose-related trends may be discerned. When values for male and female groups are considered together only 3 parameters are seen to vary significantly from control values. Rats fed

with the 10% diet have significantly lower urea concentrations, the 10% and 20% groups have significantly higher sodium concentrations and the 5% groups have lower alanine aminotransferase levels than control animals. Since dose-related trends are not seen in relation to these it is likely that they do not reflect toxic effects of gum arabic. Furthermore it is of interest that no alteration in albumin or total protein concentration can be seen, suggesting that while somatic protein indirectly reflected by total body weight decreased with increasing gum dosage, visceral protein concentrations are unaffected.

Caecal size increases relative to body weight were universally noted, particularly in the 5 and 10% gum arabic fed animals and this size increase is significant in the 10% animals when compared to control values.

Pathological abnormalities at post mortem were few and no differences between controls and any of the treatment groups are noted.

TABLE 1

Final animal weights after ingestion of O.B.D. or O.B.D. + G.A. diets for two years.

% G.A. in diet	0%	2.5%	5%	10%	20%
MALE	n = 4 459.6 ± 11.8	n = 3 439.1 ± 6.6	n = 5 444.9 ± 23.4	n = 5 439.9 ± 24.9	n = 5 ^a 409.3 ± 10.9
FEMALE	n = 4 266.5 ± 27.9	n = 5 254.4 ± 9.3	n = 4 243.2 ± 19.8	n = 3 243.0 ± 10.5	n = 5 ^b 232.9 ± 9.6

a $p < 0.001$

b $p < 0.05$

Treated vs. controls

TABLE 2

Haematological indices in male and female rats fed O.B.D. or O.B.D. + G.A. for two years (mean \pm S.D.).

		MALES						
% G.A. in diet		$\times 10^9/1$ WBC	$\times 10^{12}/1$ RBC	gm/dl. Hb	Hct	fl MCV	pg MCH	gm/dl. MCHC
0	n=4	9.7 \pm 3.59	8.28 \pm .09	16.2 \pm .14	.455 \pm .007	55.5 \pm 1	19.9 \pm .2	35.8 \pm .33
2.5	n=3	6.1 \pm .23	8.22 \pm .15	16.2 \pm .27	.449 \pm .01	56 \pm .58	19.9 \pm .17	36.2 \pm .27
5.0	n=5	6.7 \pm 1.48	8.13 \pm .5	16.2 \pm .67	.441 \pm .02	55 \pm .71	20.3 \pm .56	36.8 \pm .9
10.0	n=4	7.55 \pm 1.47	8.33 \pm .4	16.5 \pm 1	.453 \pm .02	55 \pm .5	20.2 \pm .31	36.3 \pm .4
20.0	n=5	8.56 \pm 1.1	8.35 \pm .25	16.1 \pm .62	.449 \pm .01	55 \pm .55	19.6 \pm .41	35.8 \pm .47
		FEMALES						
		WBC	RBC	Hb	Hct	MCV	MCH	MCHC
0	n=4	6.6 \pm 1.2	7.58 \pm .26	15.2 \pm .31	.421 \pm .01	56 \pm .96	20.3 \pm .28	36.1 \pm .19
2.5	n=5	7.8 \pm 1.2	7.52 \pm .28	14.9 \pm .59	.415 \pm .02	56 \pm .55	20.2 \pm 20	35.9 \pm .22
5.0	n=3	6.9 \pm .67	7.2 \pm .36	14.3 \pm .49	.398 \pm .02	56 \pm .58	20.3 \pm .35	36.1 \pm .8
10.0	n=2	7.85 \pm .29	7.64 \pm .18	15.2 \pm .5	.418 \pm .008	55 \pm -	20.2 \pm .21	36.2 \pm .35
20.0	n=4	6.15 \pm 1.19	7.45 \pm 0.22	14.6 \pm .46	.403 \pm .015	55 \pm .58	20.2 \pm .22	36.3 \pm .24

Serum biochemical indices in male and female rats fed O.B.D. or O.B.D. + G.A. for two years. (Male and female values together in Table 3B). (Mean \pm S.D.).

% G.A.	Albumin	Urea	Na ⁺	K ⁺	CO ₂	ALT	Alk.Phos.	Ca ⁺⁺	PO ₄	Creat.	Uric	AST	Tot.Prot.
n=4 0	34 _± 1.3	6.63 _± .8	145 _± 3.3	5.3 _± 1.5	21 _± 1.8	108	10.2 _± 229 _± 49	2.69 _± .13	1.98 _± .05	47 _± 1.26	.16 _± .02	194 _± 62.2	61 _± 2.16
n=3 5	34 _± 1.16	6.1 _± 2	149 _± 2	5.87 _± .23	20 _± 0	64	12 ^b _± 274 _± 66	2.65 _± .08	1.83 _± .1	43 _± 2.0	.15 _± .03	199 _± 43.6	64 _± 2.0
n=4 10	34 _± 2.58	5.7 _± .25	147 _± 2.06	6.6 _± .99	19 _± 2	18.9 _± 23	225 _± 42	2.64 _± .12	2.1 _± 2	45 _± 2.8	.19 _± .02	293 _± 73.7	64 _± 5.7
n=4 20	34 _± 1.16	8.4 _± .48 ^c	150 _± 1.5 ^d	5.8 _± .28	21 _± 1	88	35 ^b _± 205 _± 34.6	2.66 _± .02	2.12 _± .38	48 _± 8.1	.16 _± .03	221 _± 74.9	62 _± 2.83

MALES

70.

	Albumin	Urea	Na ⁺	K ⁺	CO ₂	ALT	Alk.Phos.	Ca ⁺⁺	PO ₄	Creat.	Uric	AST	Tot.Prot.
n=4 0	41±0	8.5 ±.62	145±2.16	5.8 ±.43	21.5±1.92	136 ±82	131±19.6	3 ±.005	2.03±.06	43±2.08	.16±.01	303±40.1	71 ±1.9
n=4 2.5	41±1.9	7.4±1.05	146±2.38	5.2 ±4	23 ±1.16	99.8±59	147±21.7	2.9 ±.12	1.88±.13	41±4.97	.14±.03	261±108	69.5±3
n=3 10.0	38±1.2 ^d	6.8±0.4 ^d	148±.58	4.7 ±.12	23 ±1.16	58.7±27.2	156±39	2.95±0.1	2.05±.23	35±3.61 ^d	.10±0 ^a	149±19.2 ^b	66.7±3.06
n=4 20.0	42±1.0	6.85±.34 ^b	147±2.1	4.9 ±.19	22 ±1.63	92.5±30.3	143±17.1	2.97±.12	2.15±.12	36±.96 ^b	.12±.01 ^b	237±97.0	69 ±1.16

FEMALES

Significant differences
vs. Control

- d p < 0.05
- c p < 0.01
- b p < 0.005
- a p < 0.001

% G.A.	Albumin	Urea	Na ⁺	K ⁺	CO ₂	ALT	Alk.Phos.	Ca ⁺⁺	Po ₄	Creat.	Uric	AST	Total Prot.	Bil
0 n = 8	37.5 +3.35 _	7.5 +1.16 _	145 +2.59 _	5.54 +0.89 _	21.3 +1.75 _	121.6 +56.1 _	180.1 +62.9 _	2.82 +0.19 _	2.00 +0.06 _	44.4 +3.11 _	0.16 +0.01 _	241 +76.6 _	65.3 +5.4 _	1 +0 _
2.5 n = 6	38.2 +3.4 _	7.07 +0.93 _	147 +3.03 _	5.3 +0.2 _	21.8 +2.2 _	85.5 +50.4 _	169.7 +40.9 _	2.85 +0.14 _	1.91 +0.15 _	42 +4.19 _	0.14 +0.03 _	230 +97 _	67 +4.5 _	1 +0 _
5 n = 5	37.4 +4.3 _	6.68 +0.87 _	148 +2.28 _	5.22 +0.12 _	20.4 +0.89 _	64 ^b +12.5 _	219.6 +88.1 _	2.75 +0.20 _	1.92 +0.14 _	43 +1.42 _	0.13 +0.04 _	183 +52.2 _	66.8 +4.2 _	1 +0 _
10 n = 7	35.1 +5.99 _	6.19 ^a +0.64 _	148 +1.60 _	5.8 +0.12 _	20.9 +2.79 _	76 +27.9 _	195.4 +52.6 _	2.77 +0.20 _	2.08 +0.20 _	40.7 +6.07 _	0.15 +0.05 _	231.6 +93.6 _	64.9 +4.7 _	1 +0.3 _
20 n = 8	37.8 +4.13 _	7.61 +0.90 _	149 ^a +2.61 _	5.3 +0.55 _	21 +1.51 _	90 +30.5 _	174 +41.7 _	2.83 +0.13 _	2.13 +0.26 _	42.1 +8.23 _	0.14 +0.03 _	229 +80.7 _	66.5 +4.24 _	1 +0 _

a p < 0.02
b p < 0.05

TABLE 4

Mean relative caecal weight ($\text{g} \cdot 100\text{g}^{-1}$) in female rats fed O.B.D. or O.B.D. + G.A. for two years. ($\text{g} \pm \text{S.D.}$).

% G.A. in diet	0	2.5	5.0	10.0	20.0
	n=4	n=5	n=4	n=3	n=5
Mean relative caecal weight ($\text{g} \cdot 100\text{g}^{-1} \pm \text{S.D.}$)	2.32 ± 0.3	2.35 ± 0.13	2.65 ± 0.13	3.16 ± 0.11	2.76 ± 0.32

$$r = 0.50$$

$$t = 3.5496$$

$$p < 0.005$$

TABLE 5

Findings at autopsy on rats fed O.B.D. or O.B.D. + G.A. for two years
(or at time of premature death)

<u>SEX</u>	<u>G.A. DOSE</u>	<u>PATHOLOGY</u>
M	0	Bladder Calculi
M	2.5%	Bladder Calculi
M	10.0%	Sertoli Cell Carcinoma Testis
M	2.5%	Diaphragmatic Hernia
F	2.5%	Hydrosalpinx
F	5.0%	Haematosalpinx
F	20.0%	Hydrosalpinx

NORMAL	
P.M.	
CONTROLS	TREATED
9/10	34/40
(90%)	(85%)

PREMATURE	
DEATH	
CONTROLS	TREATED
1/10	4/40
(10%)	(10%)

4 - No cause of death
apparent
1 - Diaphragmatic hernia

Chapter 3 (ii)

Effects of the sub-chronic administration of gum arabic to rats.

Introduction

This study examined the consequences of feeding a high dose of gum arabic to a group of rats over a period of 90 days. The experimental design was such as to satisfy EEC and UK requirements regarding the sub-chronic testing of ingestible substances.

Materials and methods

2 month old male and female Wistar rats (average weight 120g.) purchased from Messrs. Bantin & Kingman, Hull were used. Animals were housed three per cage under conditions described in Chapter 2. Two diets were employed: 1. Reconstituted oxoid pellets (O.B.D.); 2. Reconstituted oxoid pellets containing 5-20% w/w gum arabic (O.B.D. + GA). Animals in group 2 were weaned onto the highest dose of gum arabic by steady increases in the percentage of gum arabic incorporated into the diet (Fig.1.). This slow introduction was necessary to minimise side effects such as diarrhoea or failure to thrive as well as to maintain the gum dose per unit body weight as constant as possible. The final gum arabic dose was $14\text{g.kg}^{-1}.\text{d}^{-1}$. Animals were fed ad libitum. Food consumption per cage was continuously monitored and an average per animal calculated.

15 animals were placed in each of 4 treatment groups which were: 1. Control males (O.B.D.); 2. Treated males (O.B.D. + GA); 3. Control females (O.B.D.); 4. Treated females (O.B.D. + GA).

In the week prior to sacrifice animals were placed in metabolic cages and collections of urine (24 hr.) obtained. During the collection period animals were allowed to eat and drink ad libitum. Ames "Multistix" were used to test all urines. Samples of urine were also tested for reducing sugars using Fehlings test.

At the end of the study blood samples were obtained by retro orbital plexus puncture using light ether anaesthesia. After venepuncture animals were sacrificed by cervical dislocation and a full post mortem carried out excluding intracranial examination. The following organs were retained and fixed in formal saline:

Gastrointestinal tract: oesophagus, stomach, upper and lower small bowel, caecum, colon, liver and pancreas.

Genitourinary system: kidneys, urinary bladder, uterus/ ovaries/uterine tubes, or testes/epididymus/seminal vesicles.

Respiratory system: lungs, trachea, bronchi

Reticulo endothelial system: spleen, mesenteric lymph nodes, thymus.

Endocrine system: adrenals

Cardiovascular system: heart.

Blocks were cut from each of these organs, embedded in paraffin wax, cut and stained using haematoxylin and eosin stain.

Results:

Food Consumption

At the end of the study treated male rats had consumed 11.2% less food than their controls. Treated females consumed 8% less than their controls (Fig.1). Food consumption ($\text{g.kg}^{-1}.\text{d}^{-1}$) was similar in all groups.

Growth Pattern

Growth curves for male and female groups of rats are shown in Figs. 2 & 3. Treated males demonstrated no significant alterations in weight gain until levels of GA incorporation in the diet exceeded 10%. Thereafter growth rate was significantly reduced in treated males ($p < 0.001$). Mean weight gain in treated males was 78% compared to control males ($p < 0.01$). Final body weight was also significantly reduced ($p < 0.001$) Table 1.

Female rats demonstrated no significant differences in growth rate, final body weight, or mean weight gain.

Biochemistry:

The results of a biochemical analysis of serum removed prior to animal sacrifice are noted in Table 2. Total CO_2 was significantly reduced and urea significantly increased in treated females. No significant differences were noted between control and treated males.

Haematology:

No significant differences in haematological parameters were noted between control and treated animals (Table 3).

Urinalysis:

Ames "Multistix" examination of 24 hour urine collections at the end of 90 days showed few differences between control and treated groups. Female control rats commonly had between 300 and 1000 mg.dl^{-1} proteinuria whilst controls universally had between $30 - 100 \text{ mg.dl}^{-1}$. A similar pattern was seen in male rats though the proteinuria in controls never exceeded 300 mg.dl^{-1} . Fehlings test was negative on all urines indicating the absence of significant pentosuria.

Pathology

Sections of the aforementioned organs were examined from all animals in each treatment group.

The only abnormalities found are noted in Table 4. No evidence of inflammatory or pre-malignant histological change was noted in any of the sections. A feature of male and female rats in the treatment groups was the presence of marked caecal enlargement. No associated evidence of histological abnormality was

present.

Final carcass and organ weights

Final carcass weights are noted in Table 5. Treated males were significantly lighter than their controls ($p < 0.001$). Treated females were insignificantly different from controls.

Liver and kidney weights expressed as absolute and relative values are noted in Table 5. Both were significantly reduced in treated male animals. Mean kidney weights were significantly lower in treated females.

Summary

Gum arabic was administered to rats at high dosage over a period of 90 days. A gum arabic dose rate of approximately $14 \text{ g.kg}^{-1}.\text{d}^{-1}$ was maintained. Animals tolerated this dose rate well and all gained weight although male animals were significantly lighter at the end of the experiment.

No significant haematological, biochemical or pathological abnormalities were noted at the end of the treatment period. Kidney weight was reduced in both male and female animals and liver weight in male animals.

Caecal enlargement was noted in all animals consuming gum arabic.

No significant acute toxic effects of ingestion of large amounts of the pentosan gum arabic were found.

TABLE 1

body weight and mean weight gain in male and female rats fed O.B.D. or O. + 20% G.A.(g). Parentheses indicate significant differences from control values.

G.A. intake							Mean Weight Gain Weeks 0-13
g.kg ⁻¹ 24h ⁻¹		1	4	7	10	13	
O.B.D.	0	167.5	283.4	357.6	405.7	430.5	306.8
O.B.D. +20%G.A.	14	162.9	254.6 ^b	304.8 ^b	342.9 ^c	362.8 ^c	239.1 ^b
O.B.D.	0	153.7	201.7	238.3	246.0	243.5	124.4
O.B.D. +20%G.A.	14	152.4	195.3	226.6 ^a	233.9	244.3	125.9

a p < 0.05

b p < 0.01

c p < 0.001

Treated vs. Control

TABLE 2

Serum biochemical indices in male and female rats fed O.B.D. or O.B.D. + G.A. for 90 days. (Mean \pm S.D.).

Mean \pm S.D.	K ⁺ mmol.l ⁻¹	Na ⁺ mmol.l ⁻¹	CO ₂ mmol.l ⁻¹	Urea mmol.l ⁻¹	ALT I.U.l ⁻¹	AST I.U.l ⁻¹
CONTROL (O.B.D.) n=11	5.2 ± 0.1	140 ± 3.9	13.8 ± 1.5	7.6 ± 0.8	19 ± 3.1	157 ± 27.7
TREATED GUM (O.B.D. + G.A.) n=10	5.0 ± 0.4	139 ± 3.9	11.8 ^a ± 1.3	9.4 ^b ± 1.0	19 ± 2.9	137 ± 25.7
CONTROL (O.B.D.) n=15	5.4 ± 0.7	144 ± 3.9	13.9 ± 1.8	8.5 ± 1.5	16 ± 4.1	135 ± 31.1
TREATED GUM (O.B.D. + G.A.) n=14	5.0 ± 0.7	143 ± 1.8	13.2 ± 1.3	8.4 ± 1.0	17 ± 4.3	137 ± 33.8

b p < 0.005

a p < 0.02

TABLE 3

Haematological indices in male and female rats fed O.B.D. or O.B.D. + G.A. for 90 days (Mean \pm S.D.).

Mean \pm S.D.	$\times 10^9 \text{ litre}^{-1}$ WBC	$\times 10^{12} \text{ litre}^{-1}$ RBC	g. dl^{-1} Hb	Hct	f1 MCV	pg MCH	g. dl^{-1} MCHC
Female							
Control (O.B.D.)	7.3 ± 0.55	6.88 ± 0.03	14.04 ± 0.67	0.375 ± 0.02	55 ± 1.2	20.5 ± 0.58	37.4 ± 0.22
Test Treated (O.B.D. + 20% G.A.)	8.25 ± 1.46	6.93 ± 0.23	13.9 ± 0.47	0.370 ± 0.004	53.8 ± 1.71	20.2 ± 0.16	37.6 ± 1.12
Male							
Control (O.B.D.)	11.9 ± 2.17	8.03 ± 0.31	15.1 ± 0.48	0.402 ± 0.013	50.9 ± 2.1	19.0 ± 0.74	37.6 ± 0.57
Test Treated (O.B.D. + 20% G.A.)	10.5 ± 1.98	7.82 ± 0.39	15.0 ± 0.47	0.396 ± 0.012	51.5 ± 2.01	19.3 ± 0.81	37.8 ± 0.60

TABLE 4

Details of pathological abnormalities found at autopsy on male and female rats fed O.B.D. or O.B.D. + G.A. for 90 days.

<u>Pathological Abnormality</u>	<u>No. of Animals</u>	<u>Sex</u> —	<u>Diet</u> —
Mild focal Pyelonephritis	1	Male	O.B.D.
Benign pancreatic adenoma	1	Male	O.B.D.
Multiple small bowel diverticula	1	Female	O.B.D.
Focal small bowel diverticula	2	1 Female 1 Male	O.B.D. O.B.D. + G.A.

TABLE 5

ass weight, liver and kidney weights of male and female rats fed O.B.D. or
D. + G.A. for 90 days.

Mean + S.D.	Final animal weight (g+S.D.)	Liver weight (g+S.D.)	Liver weight g.100g ⁻¹ animal weight +S.D.	Kidney weight (g+S.D.)	Kidney weight g.100g ⁻¹ animal weight + S.D.
(O.B.D.) n = 15	248.8 +15.65	9.55 +0.72	3.85 +0.33	2.31 +0.29	0.93 +0.09
(O.B.D. + 20% G.A.) n = 15	245.9 +13.8	9.34 +1.01	3.80 +0.38	1.94 ^a +0.12	0.79 ^a +0.05
(O.B.D.) n = 15	432.3 +35.54	15.42 +1.50	3.57 +0.24	3.97 +0.50	0.93 +0.11
(O.B.D. + 20% G.A.) n = 15	364.5 ^a +31.58	11.62 ^a +1.38	3.20 ^b +0.36	3.09 ^a +0.37	0.85 ^d +0.08

Treated vs. Control

a $p < 0.001$

b $p < 0.005$

c $p < 0.01$

d $p < 0.05$

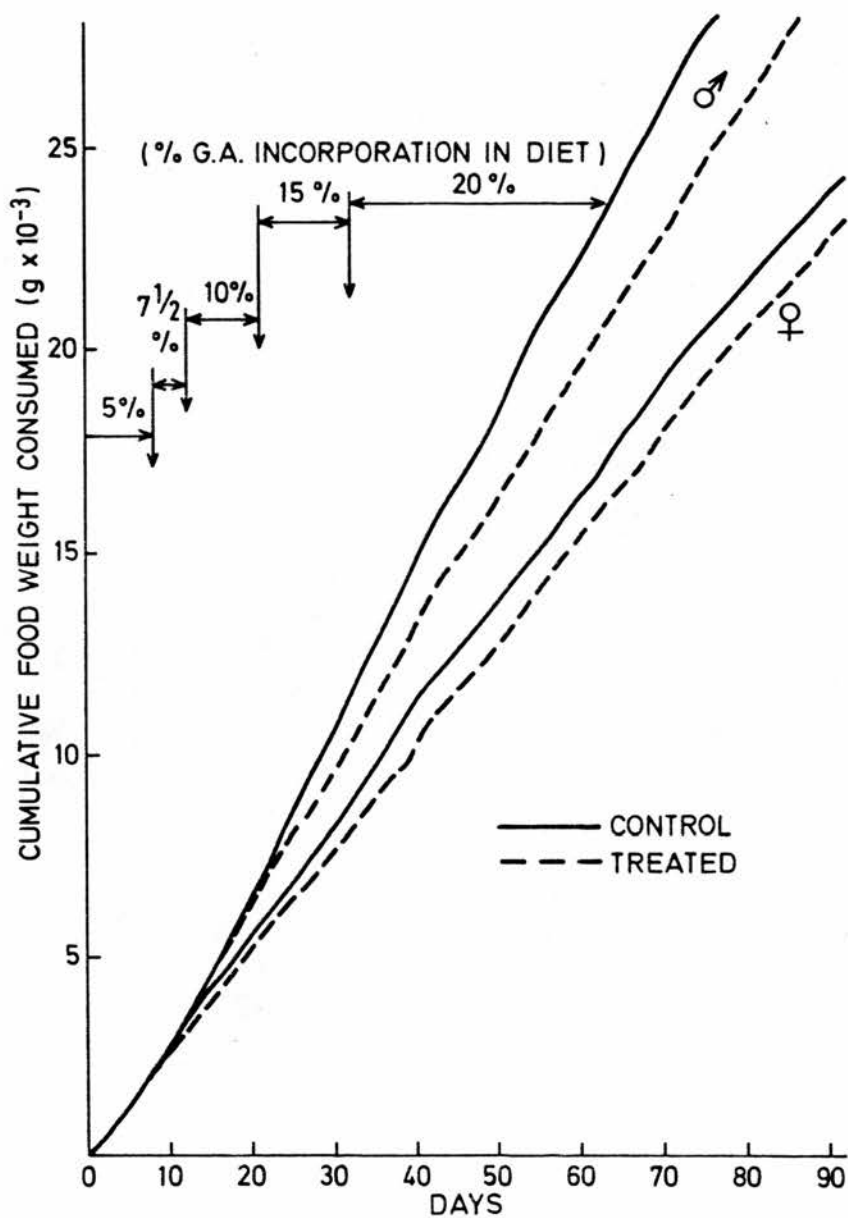


Fig.1: Food consumption in male and female rats fed O.B.D. (control) or O.B.D. + G.A. (treated) for a period of 90 days.

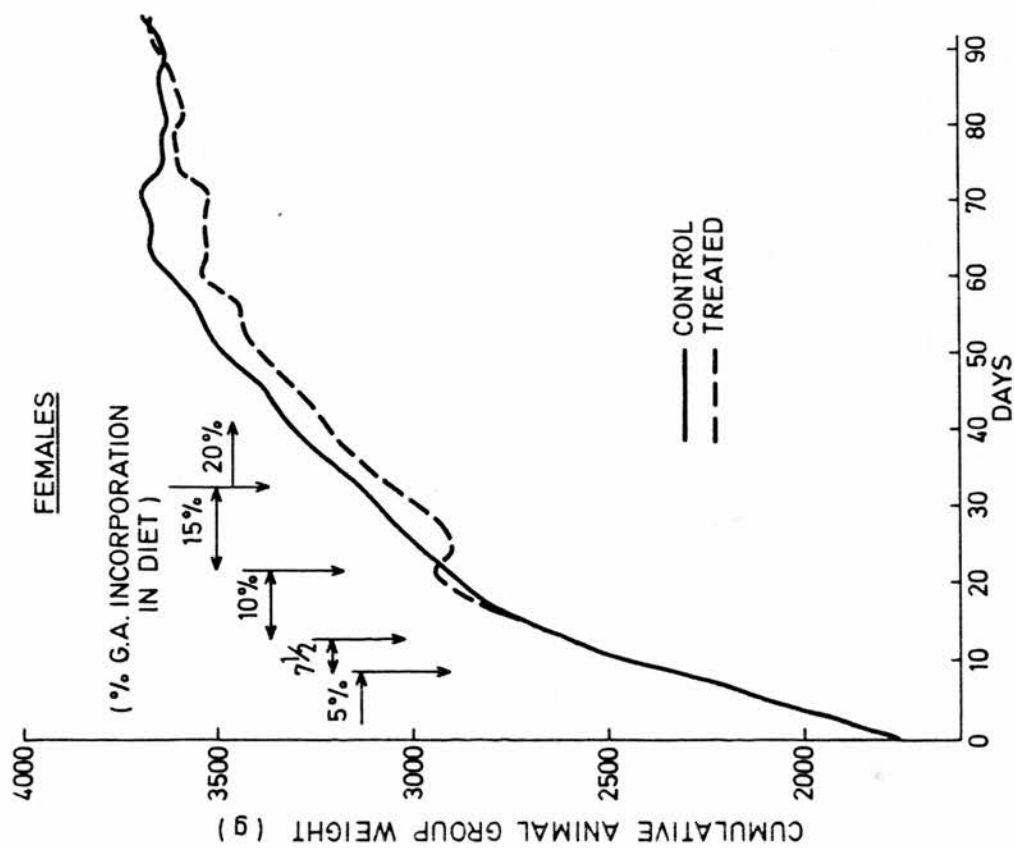


Fig.2: Weight gain in female rats fed O.B.D. (control) or O.B.D. + G.A. (treated) for 90 days.

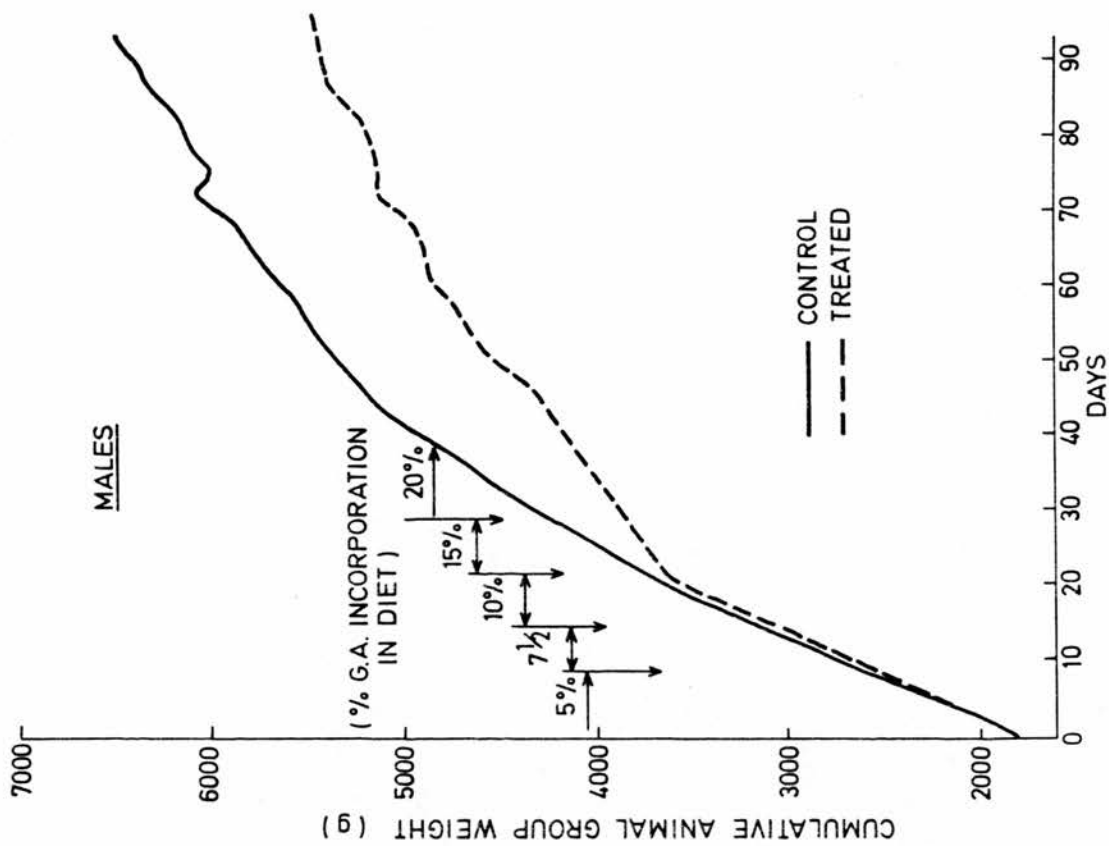


Fig.3: Weight gain in male rats fed O.B.D. (control) or O.B.D. + G.A. (treated) for 90 days.

Chapter 3 (iii)

The effects on growth of feeding various dietary regimens containing gum arabic to adult rats in short term experiments.

The effect of feeding high doses of gum arabic to young rats has been referred to in Chapter 3(ii). To assess the nutritional adequacy of various diets containing gum arabic these were fed to adult male Wistar rats. Diet was administered in one of two forms.

1. A reconstituted pellet diet: O.B.D. or O.B.D. + G.A.
2. A nutritionally complete elemental diet: Elem-D or Elem-D + G.A.

Pellet diet

Male albino Wistar rats (c. 300g.) were fed O.B.D. or O.B.D. + G.A. ad libitum for 26 days. G.A. dosage in the O.B.D. + G.A. groups was 2.5%, 5.0%, 10.0% and 20.0% respectively. Animals were weighed at 6 day intervals and food weight consumed recorded. Animals were not weaned on to the diets in these experiments.

Results

Dietary intake is noted in Table 1. Animals in the 10 & 20% G.A. groups consumed significantly less energy (-12%; -35.5%) ($p < 0.005$; $p < 0.001$ respectively), protein (-11.6%, -35.8%) ($p < 0.005$; $p < 0.001$) and fibre (11.6%; -34.8) ($p < 0.005$, $p < 0.001$) than controls. Animals on O.B.D. + 2.5% G.A. diet consumed greater amounts of protein, energy and fibre than controls though these differences were insignificant. O.B.D. + 5% animals consumed greater amounts of protein and energy than controls (+6.0%, + 7.0%) ($p = \text{N.S.D.}$).

Growth curves are shown in Figs. 1 & 2. All animals gained weight during the experiment apart from three rats in the O.B.D. + 20% G.A. group whose weight fell. Mean percentage weight increases are noted in Table 2. O.B.D. + 20% G.A. animals gained significantly less weight and O.B.D. + 5% G.A. significantly more weight than control.

Elemental diet

Male albino Wistar rats (c. 300g) were fed Elem-D or Elem-D + 13% G.A. ad libitum for 16 days. The diet was presented in the form of moulds each weighing approximately 20-25g. Each tub of Elem-D + G.A. contained approximately 1.3g G.A. Animals were offered 3 tubs per day during the experiment. Because of the nature of the diet quantification of the amount eaten was difficult but it is unlikely that wastage exceeded 25%. Gum arabic dose was therefore approximately $3\text{g}\cdot\text{day}^{-1}\cdot\text{animal}^{-1}$.

Results

Weight gain curves are noted in Figs. 3. Both Elem-D and Elem-D + G.A. animals gained weight. Mean percentage weight gain was slightly lower in the Elem-D + G.A. group but the difference was statistically insignificant. Weight gain per day was very similar to that on O.B.D. + G.A. diets. (Table 3).

TABLE 1

Dietary analysis of male rats fed O.B.D. or O.B.D. + G.A. (Mean + S.D.). Parentheses indicate significant differences between control (0%) values and the figure indicated in the same vertical column. Estimated values for Elem-D and Elem-D + G.A. are noted below.

G.A. dose (% w/w)	Mean body weight g.	Mean Food Intake -1 g.kg ⁻¹ .24h	Mean Protein Intake -1 g.kg ⁻¹ .24h	Mean Energy Intake -1 mJ.kg ⁻¹ .24h	Mean Fibre Intake -1 g.kg ⁻¹ .24h	Mean G.A. Intake -1 g.kg ⁻¹ .24h
0	5 340.3 ± 20.5	70.3 ± 3.8 ^{ab}	2.5 ± 0.13 ^{ab}	0.84 ± 0.05 ^{ab}	2.5 ± 0.13 ^{ab}	-
2.5	5 362 ± 24.5	71.5 ± 3.6	2.54 ± 0.13	0.85 ± 0.04	2.55 ± 0.13	1.8 ± 0.09
5	5 315.9 ± 33.0	74.5 ± 8.5	2.65 ± 0.3	0.9 ± 0.1	2.48 ± 0.71	3.9 ± 0.43
10	5 329.1 ± 10.4	62.0 ± 2.3 ^a	2.21 ± 0.08 ^a	0.74 ± 0.03 ^a	2.21 ± 0.08 ^a	6.9 ± 0.3
20	5 315.8 ± 14.3	45.8 ± 3.7 ^b	1.63 ± 0.13 ^b	0.55 ± 0.04 ^b	1.63 ± 0.13 ^b	11.5 ± 0.9
Elem-D	5 347 ± 11.2	-	4.3	0.88	-	-
Elem-D + 13%	5 322 ± 19.1	-	5.1	1.03	-	3

a p < 0.005
b p < 0.001

TABLE 2

Percentage weight gain in rats fed O.B.D. or O.B.D. + G.A. for 26 days (% \pm S.D.). Significant differences in weight gain from controls (0%) are noted in parentheses.

G.A.	O.B.D.	O.B.D. + G.A.			
	0	2.5%	5.0%	10.0%	20.0%
Mean % weight gain	21	19	27 ^b	18	2.0 ^a
\pm S.D.	\pm 3.1	\pm 5.2	\pm 1.5	\pm 3.7	\pm 7.9

a $p < 0.001$

b $p < 0.01$

TABLE 3

Weight gain in male rats fed Elem-D or Elem-D + 13% G.A. for 16 days (Mean \pm S.D.).

	Elem-D	Elem-D + G.A.
Mean % weight gain	17.6	15.3
\pm S.D.	\pm 8.2	\pm 3.8

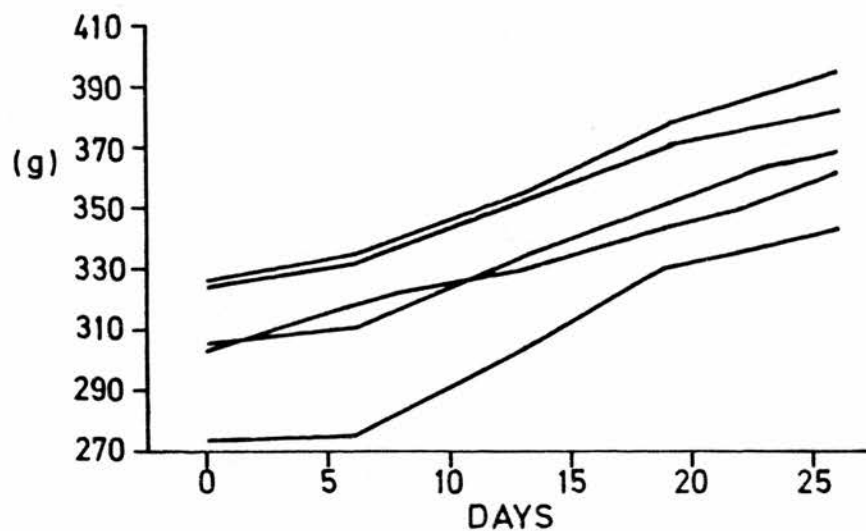


Fig.1: Growth curves of male rats fed O.B.D. ad libitum for 26 days.

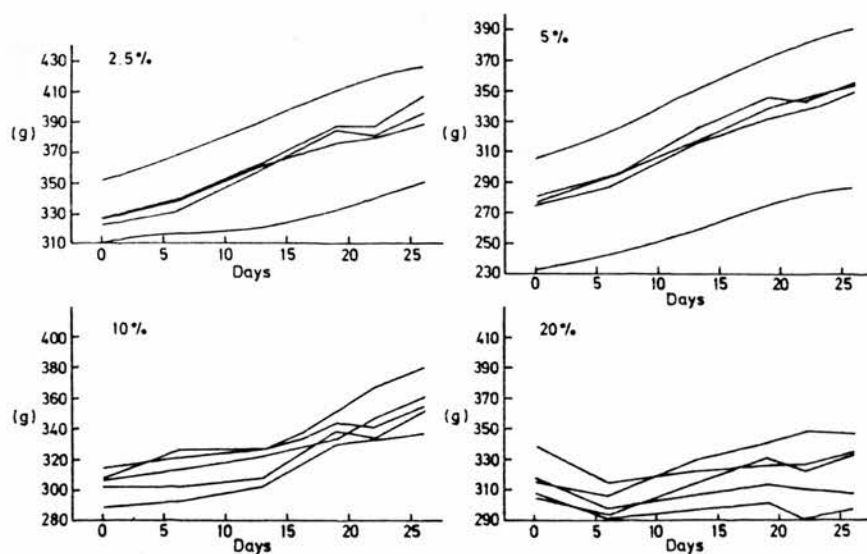


Fig.2: Growth curves of male rats fed O.B.D. + 2.5, 5.0, 10.0 and 20.0% G.A. for 26 days.

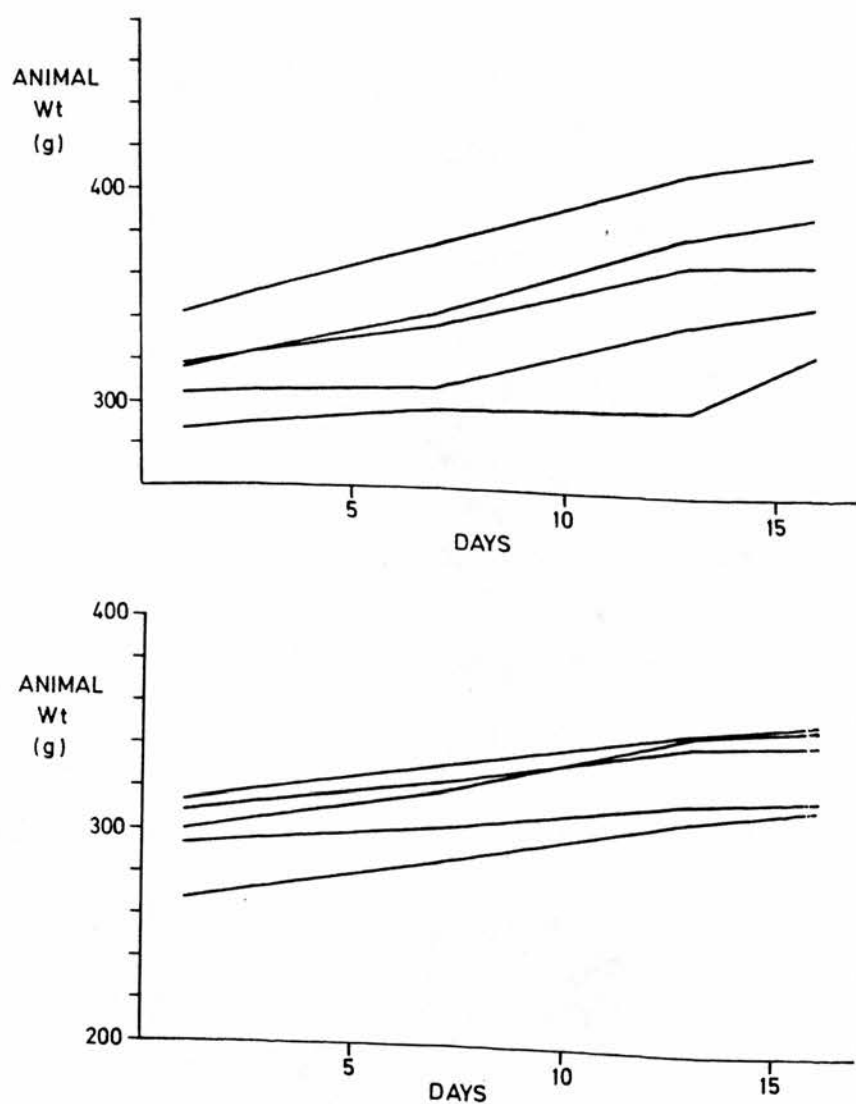


Fig.3: Growth curves of male rats fed Elem-D (above) or Elem-D + G.A. (below) for 16 days.

Chapter 3 (iv)

Effects of varying dietary regimens on faecal and caecal weight in the rat.

The effect of G.A. on faecal output was measured in two situations.

1. In animals fed a nutritionally complete reconstituted oxoid pellet diet (O.B.D.; O.B.D. + 10% G.A.)
2. In animals fed a nutritionally complete elemental diet (Elem-D + G.A.)

Pellet diet

Male albino Wistar rats (c. 400g) were fed a reconstituted oxoid pellet diet O.B.D. ad libitum for 10 days. A 24 hour collection of faeces was taken on the 10th day. Diet was available ad libitum during the collection period. Grid floored cages were used during the collection in an attempt to minimise coprophagia. After this collection animals were offered O.B.D. + 10% G.A. ad libitum for 2 weeks. At the end of this period a further 24 hr. collection was taken. Animal weights and ingested food weights were recorded during the periods of study.

Faeces were freeze dried and dry faecal weight measured as noted previously. At the end of the study the animals were sacrificed, the caecum removed and its contents weighed.

Results

Table 1 shows the results for dry and wet faecal weights in relation to animal weight and dietary factors. Wet weights are probably underestimates due to faecal dessication prior to collection. Dry weight increased by 32% on O.B.D. + 10% G.A. ($p < 0.0025$). This may partly be explained by increased food and fibre ingestion (30% and 16% respectively). Average G.A. intake per animal was $3g \cdot d^{-1}$.

Elemental diet

Male albino Wistar rats (c.300g) were offered a nutritionally complete elemental diet ad libitum for 16 days. At the end of the study period a 72 hour collection was made with the rats in grid floored cages to discourage coprophagia. Faeces were collected, frozen (-20°C) and then freeze dried. Only dry weights were recorded as considerable faecal drying took place prior to collection due to the relatively unformed nature of the stools. After the faecal collection the animals were sacrificed, the caecum removed, and its contents weighed.

Results

Having allowed for approximately 25% scattering daily ingested dose of G.A. was c.3g.

Faecal weights are noted in Table 2. Both Elem-D and Elem-D + G.A. resulted in marked reductions in faecal output when compared to faecal output in O.B.D. or O.B.D. + G.A. Faecal weight was significantly higher ($p < 0.02$) in rats on Elem-D + G.A. although output was still small compared to that on O.B.D. Despite an intake of at least 3g.d^{-1} G.A. mean daily faecal dry weight was only $1.0 \pm 0.3 \text{ g.d}^{-1}$.

Effects of pellet and elemental diet on caecal weight

The administration of elemental diet (Elem-D) resulted in a significant reduction in relative weight of caecal content ($p < 0.001$). As noted in chronic administration addition of G.A. to the diet caused marked increases in caecal weight after O.B.D. + G.A. ($p < 0.01$) and for elemental diet ($p < 0.001$). The proportional rise for elemental (308%) is much greater than that for pellet diet (61%). Furthermore caecal dry weight was significantly greater when Elem-D + G.A. was administered than after O.B.D. + G.A. ($p < 0.05$).

TABLE 1

ly faecal weight and dietary intake in male rats given O.B.D. and O.B.D. + 10% G.A. ts (mean \pm S.D.). Figures with common superscripts are significantly different.

	Final Body Weight g.	Faecal Wet Weight g.d ⁻¹	Faecal Dry Weight g.d ⁻¹	Food Intake g.kg ⁻¹ .d ⁻¹	Fibre Intake g.kg ⁻¹ .d ⁻¹	Gum Intake g.kg ⁻¹ .d ⁻¹
.D. 10	443 \pm 22	9.3 \pm 2.5 ^a	5.9 \pm 1.8 ^b	51.9 \pm 5.2	1.9 \pm 0.3	-
.D. + G.A. 10	449 \pm 26	13.5 \pm 2.4 ^a	7.8 \pm 1.1 ^b	67.2 \pm 4.5	2.2 \pm 0.1	6.7

a $p < 0.001$

b $p < .0025$

TABLE 2

ELEMENTAL DIET

ly faecal weight of rats on Elem-D or Elem-D + 13% G.A. Only dry faecal weight quoted since faecal dessication prior to collection make wet weights considerable overestimates (mean \pm S.D.). Figures with common superscripts are significantly different.

	Final Body Weight (g)	Faecal Dry Weight (g)
Elem-D n = 9	330 \pm 13	0.7 ^a \pm 0.2
Elem-D + 13% G.A. n = 9	311 \pm 9	1.0 ^a \pm 0.3

a $p < 0.02$

TABLE 3

Relative caecal weight ($\text{g} \cdot 100\text{g}^{-1}$) of rats fed pellet or elemental diets with and without gum supplements (mean \pm S.D.). Figures with common superscripts are significantly different.

	O.B.D.	O.B.D. + 10% G.A.	Elem-D	Elem-D +13% G.A.
	n = 5	n = 10	n = 5	n = 5
Caecal weight $\text{g} \cdot 100\text{g}^{-1}$	2.3 ^{a,c,d} ± 0.3	3.7 ^c ± 0.8	1.2 ^{b,d} ± 0.3	4.9 ^{a,b} ± 1.1

a $p < 0.05$

b $p < 0.001$

c $p < 0.01$

d $p < 0.0001$

Due to the design of this study caecal weights clearly were not available during O.B.D. administration and this figure is extracted from values obtained for caecal weight after administration of O.B.D. in Chapter 3(i).

Chapter 3 (v)

The presence of G.A. in the intestinal content of the rat

The intestinal content of rats fed G.A. was examined to determine the presence of gum arabic at various points in the gut in an attempt to determine whether and if so where G.A. was degraded during intestinal transit, G.A. being recognised by its property of precipitating in acidic ethanol solution.

Method

Three month old male Wistar rats (c.350g) were fed O.B.D. + 20% G.A. or O.B.D. for 4 weeks ad libitum. After overnight feeding rats were sacrificed by a combination of ether anaesthesia and cervical dislocation and the intestine from stomach to distal colon removed. The contents of the following organs were removed and placed in separate plastic containers: stomach, small bowel, caecum and distal colon (the distal half of the removed colon). In addition the faeces passed in the 24 hours period prior to sacrifice were collected.

Intestinal contents were diluted in water (1:2 content/water) and then mixed for 10 minutes to allow G.A. present fully to dissolve. The resulting suspension was bench centrifuged for 10 mins. at 3,000 rpm and then in a high speed centrifuge at 9160g to remove suspended material. The supernatants were subjected to acid ethanol (95%) precipitation.

Results

Control diet (O.B.D.): at all sites no precipitate typical of G.A. was obtained, however a gelatinous precipitate did appear and this was retained for hydrolysis.

O.B.D. + 20% G.A.: a precipitate typical of G.A. was obtained from stomach and small intestinal content but could not be found from caecal and colonic content or faeces (Fig.1). Although no typical G.A. precipitate was obtained in caecal and

colonic content and faeces a gelatinous and meagre precipitate formed very similar to that demonstrated in O.B.D. rats (Fig.2) and this was retained for hydrolysis and chromatography.

The effect of caecal excision

Male Wistar rats previously subjected to caecal excision as described previously (pp 55) and allowed to recover over a 2 months period were fed O.B.D. + 20% G.A. ad libitum for 2 weeks and the animals then sacrificed. Intestinal contents were collected as before including the faeces passed by the animals in the 24 hours prior to sacrifice. Suspension of the intestinal contents and faeces were made as before and ethanolic precipitation performed.

Results

During the initial stages of feeding O.B.D. + 20% rats developed diarrhoea and although this had settled by the end of the feeding period the stools passed were still subjectively more loose and less well formed than when on O.B.D.

A precipitate typical of G.A. was obtained from all levels of the intestine Table 1 as well as from faeces Fig.3.

Hydrolysis and chromatographic assessment of precipitates

Each precipitate was hydrolysed in the manner described in Chapter 2 pp 51 and the resultant hydrolysate examined using paper and thin layer chromatography. Results were unsatisfactory. The hydrolysate of the gelatinous precipitate from caecal and colonic contents of O.B.D. and O.B.D. + G.A. rats demonstrated the presence of multiple sugars making individual identification impossible. The white flocculent precipitate from stomach and small bowel of O.B.D. + G.A. fed rats while demonstrating sugars of Rf values corresponding to Gal, Ara, Rha also demonstrated other sugars. Multiple dissolution and precipitation of this

precipitate did produce some clarification of the G.A. pattern though admixture was still a problem making chromatographic confirmation of G.A. unreliable.

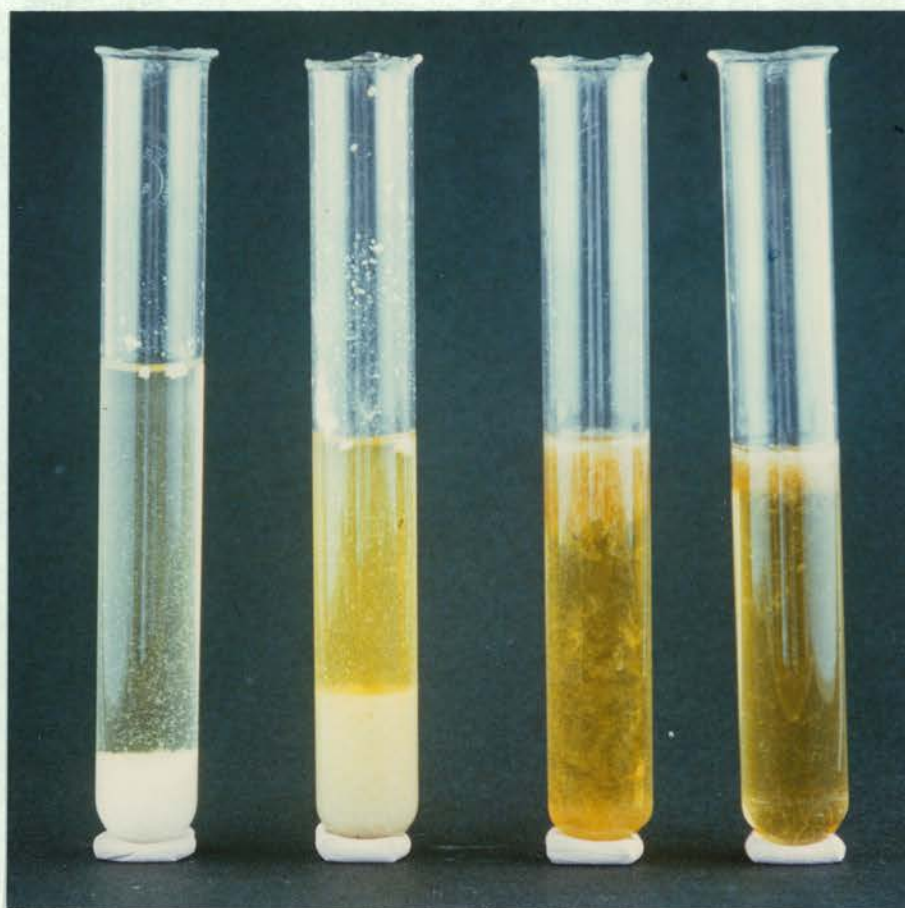


Fig.1: Precipitates obtained from centrifuged aqueous suspensions of intestinal content (L to R stomach, small bowel, caecum, distal colon) after addition of acidified ethanol (95%), from rats fed O.B.D.+ 20% G.A.



Fig.2: The meagre gelatinous precipitate obtained from the centrifuged aqueous suspensions of faeces from rats on O.B.D. (Left) and O.B.D. + 20% G.A. (Right) after addition of acidic ethanol (95%).

Fig.3: The appearance of a G.A. precipitate in the centrifuged aqueous suspension of faeces from a caeectomised rat fed O.B.D. + 20% G.A. (acidic-ethanol precipitation) (black and white).

Chapter 3 (vi)

Volatile fatty acid production in rats fed G.A.

Pellet diet

Male albino Wistar rats were fed O.B.D. or O.B.D. + G.A. (2.5%, 5.0%, 10.0%, 20.0% w/w) ad libitum for 4 weeks. At 4 weeks a faecal collection (24 hr.) was made, the faeces then frozen (-20°C) and then freeze dried. V.F.A. concentrations in samples of freeze dried faeces were measured by the G.L.C. technique described earlier (pp 52). The food weight consumed and animal weights were recorded during the study period.

Results

Food, fibre, and gum intake are noted in Table 1 Chap. 3.(iii). Despite rats in the O.B.D. + 20% G.A. group consuming less of the diet than other groups, G.A. ingestion per kg. body weight increased with increased incorporation of G.A.

Fig. 1 demonstrates the effect of G.A. on the concentration of V.F.A. in the faeces. A relationship exists between G.A. dose and the faecal V.F.A. concentrations ($r=0.61$ $p < 0.01$). Table 1 shows that daily output of V.F.A. also increased with increasing intake of G.A. in a pellet diet. V.F.A. output was slightly lower than in the control group but the difference is insignificant. Fig. 2 demonstrates the relationship between ingested dose of G.A. and the contribution of acetate and butyrate to total faecal V.F.A. concentration. Increasing G.A. consumption resulted in a linear increase in acetate and decrease in butyrate proportion ($r=0.89$ & 0.96 respectively). Propionate proportion demonstrated no consistent relationship with G.A. dose.

When compared with the proportions in control diet butyrate proportions were significantly decreased in O.B.D. + 2.5, 5.0, 10.0 and 20.0% G.A. groups ($p < 0.02$, $p < 0.02$, $p < 0.005$ and $p < 0.001$ respectively).

The effect of caecectomy on faecal V.F.A.

Three rats previously subjected to caecal excision and allowed to recover for two months were given O.B.D. + 20% G.A. ad libitum. At the end of four weeks a 24 hour collection was made and freeze dried as before.

Results

Faecal V.F.A. concentrations were significantly reduced when compared to those in the faeces of animals with an intact G.I. tract fed O.B.D. + 20% G.A. ($p < 0.01$). Faecal output was also markedly reduced (Table 2). Interestingly V.F.A. levels from caecectomised rats on O.B.D. + 20% G.A. were similar to those from intact animals on O.B.D.

V.F.A. concentrations in intestinal contents of rats on G.A.

Male albino Wistar rats were allowed O.B.D. or O.B.D. + 10% G.A. ad libitum for 2 weeks. Rats were then sacrificed and intestinal contents removed and immediately frozen prior to freeze drying and storage. V.F.A. was measured as noted previously.

Results

The elution pattern of a standard solution of the six naturally occurring V.F.A. detected is noted in Fig.3. Fig.4 demonstrates the typical V.F.A. profile seen in the rat intestine. Little or no V.F.A. is detectable in the small bowel content. The caecum contains the highest concentration of V.F.A., the concentration falling as the distal colon is reached. Faecal levels are lower than those in the distal colon

The elution pattern in Fig.5 shows the typical V.F.A. profile on O.B.D. Acetate, propionate, and butyrate are the main contributors with an inconstant and minor contribution from valerate. This pattern does not alter when G.A. is added to the

diet in pellet form (O.B.D. + G.A.)

The caecal colonic and faecal concentrations of total V.F.A. in rats on O.B.D. or O.B.D. + 10% G.A. are recorded in Fig.6. A stepwise reduction in V.F.A. concentrations was seen from the caecum distally in both control and gum supplemented rats. Furthermore the gum supplemented (O.B.D. + G.A.) intestinal contents have higher mean V.F.A. concentrations at each site compared to controls. There was however much overlap of concentrations between the two groups and only in faeces was there a significant difference between O.B.D. & O.B.D. + 10% ($p < 0.01$). When total V.F.A. content was derived from weight of intestinal content and V.F.A. concentration the O.B.D. + 10% group showed significantly higher levels ($p < 0.05$) of V.F.A. in the caecum than rats on O.B.D. (Table 3). No significant differences were present in colonic V.F.A. content or faecal V.F.A. output. The ready emptying of the distal colon consequent upon rat handling results in inconstant and unreliable values. The rat caecum, however, assuming similar overnight feeding patterns remains relatively constantly filled and thus total V.F.A. content may be more readily compared.

The proportions of V.F.A. present at each site in the intestine are noted in Table 4. In both O.B.D. & O.B.D. + G.A. groups higher proportions of acetate and lower proportions of butyrate were seen in faeces than in caecal contents. A stepwise reduction in butyrate and increase in acetate proportion was seen from caecum to colon to faeces. Propionate also demonstrated a stepwise increase in proportion from caecum to faeces, but only for O.B.D. diet.

The influence of elemental diet on V.F.A. production in the rat intestine.

Male albino Wistar rats (c.300g) were given Elem-D or Elem-D + 13% G.A. ad libitum for 16 days. Animal weights were recorded. At the end of 16 days a 3 day faecal collection was

taken and the rats then sacrificed. Intestinal contents were removed after evisceration, frozen immediately and then freeze dried.

Results

The elution patterns of V.F.A. in caecal contents from animals on Elem-D or Elem-D + 13% G.A. are displayed in Fig.7. Elem-D + 13% G.A. caecal content and faeces had a similar pattern to that seen on O.B.D. diet. However caecal content contained small quantities of isovaleric, valeric, and isobutyric acid when Elem-D was administered.

Caecal V.F.A. concentration, V.F.A. contents, faecal concentrations and outputs are noted in Table 5. Estimations of colonic V.F.A. content were not attempted in view of the minimal volume whilst on elemental diets.

Caecal V.F.A. concentrations were significantly less in the Elem-D + G.A. group ($p < 0.005$), a reverse of the findings in rats fed O.B.D. + 10% G.A. The total quantity of V.F.A. in the caecum was, however, higher in the Elem-D + 13% G.A. group. Faecal V.F.A. concentration was significantly higher on Elem-D + 13% G.A. and isomeric forms persisted in the faecal V.F.A. profile of Elem-D rats. Daily output of V.F.A. rose on Elem-D + 13% G.A. but the increase was insignificant.

As in rats on O.B.D./O.B.D. + G.A. acetate proportions rose and butyrate proportions fell between caecal contents and faeces on both elementary dietary regimens. However, the butyrate/propionate ratio seen on O.B.D./O.B.D. + G.A. diet was reversed when Elem-D/Elem-D + G.A. was administered. (Table 6).

Butyrate proportions were particularly reduced on Elem-D. Part of this reduction was due to the appearance of isomeric forms and valerate on Elem-D these amounting to approximately 15% of total V.F.A. Valerate and isomeric forms were absent in Elem-D + 13% G.A. faeces and caecal contents.

Elemental diet after caecectomy.

Attempts were made to feed Elem-D and Elem-D + G.A. to a further group of rats which had undergone caecectomy. These diets resulted in unremitting severe diarrhoea, particularly on Elem-D + G.A. with a subsequent deterioration in health of the animals which forced the abandonment of this study.

TABLE 1

Mean faecal VFA output ($\text{mg} \cdot 24\text{h}^{-1}$) in rats fed O.B.D. or O.B.D. + G.A. (mean \pm S.D.).

% G.A.		0	2.5	5.0	10.0	20.0
G.A. dose	$\text{g} \cdot \text{kg}^{-1} \cdot 24\text{h}^{-1}$	0	1.84	3.94	6.9	11.5
	\pm S.D.		± 0.09	± 0.43	± 0.30	± 0.90
Mean Faecal VFA		79.7	49.4	53.7	96.3	100.8
$\text{mg} \cdot 24\text{h}^{-1} \pm$ S.D.		± 39.1	± 19.2	± 16.0	± 14.9	± 40.8

TABLE 2

Mean faecal VFA concentration ($\text{mg} \cdot \text{g}^{-1}$) and output ($\text{mg} \cdot 24\text{h}^{-1}$) in intact and caeectomised rats fed O.B.D. + 20% G.A. (mean \pm S.D.).

	<u>Intact Rats</u>	<u>Caecectomy Rats</u>
	n = 5	n = 3
V.F.A. Concentration	16.9 ^a	6.9 ^a
$\text{mg} \cdot \text{g}^{-1}$	± 4.3	± 1.4
V.F.A. output	100.8	55.9
$\text{mg} \cdot 24\text{h}^{-1}$	± 40.8	± 0.4

^a $p < 0.01$ (intact vs. caeectomy)

TABLE 3

V.F.A. content of caecum and distal colon and daily faecal V.F.A. output of rats fed O.B.D. or O.B.D. + 10% G.A. (mg. \pm S.D. n = 5 for each group).

	Mean caecal V.F.A. content mg. \pm S.D.	Mean colonic V.F.A. content mg \pm S.D.	Mean faecal V.F.A. output mg.24h ⁻¹ \pm S.D.
et O.B.D. (n = 5)	31.19 \pm 5.05	15.87 \pm 4.16	10.66 \pm 2.00
O.B.D. + 10% G.A. (n = 5)	41.30 \pm 6.59 ^x	27.24 \pm 11.93	18.05 \pm 9.78
^x p < 0.05			

TABLE 4

Proportions of individual V.F.A.s in faeces, caecal and colonic contents of rats fed O.B.D. or O.B.D. + 10% G.A. Figures with common superscripts are significantly different (mean % \pm S.D.).

	CAECAL CONTENT		COLONIC CONTENT		FAECES	
	O.B.D.	O.B.D. + 10% G.A.	O.B.D.	O.B.D. + 10% G.A.	O.B.D.	O.B.D. + 10% G.A.
acetate	52.9 ^a \pm 7.0	60.5 ^d \pm 6.5	55.0 \pm 4.2	61.5 \pm 12.2	66.4 ^a \pm 2.2	68.4 ^d \pm 2.2
propionate	12.5 ^b \pm 1.6	12.4 \pm 2.4	15.2 \pm 8.4	12.1 ^f \pm 1.6	16.6 ^{b.g} \pm 0.7	14.2 ^{g.f} \pm 1.0
butyrate	34.6 ^c \pm 6.7	27.1 ^c \pm 4.9	29.9 \pm 6.6	26.4 \pm 12.9	16.9 \pm 2.1	17.5 ^e \pm 1.8
acetate: propionate	4.2	4.9	3.6	5.1	4.0	4.8
acetate: butyrate	1.5	2.2	1.8	2.3	3.9	3.9
propionate: butyrate	0.4	0.5	0.5	0.5	1.0	0.8
^a p < 0.005 ^d p < 0.05 ^g p < 0.005 ^b p < 0.001 ^e p < 0.005 ^c p < 0.001 ^f p < 0.05						

TABLE 5

Caecal V.F.A. concentration and content, faecal V.F.A. concentration and output in rats fed Elem-D or Elem-D + 13% G.A. Figures with common superscripts are significantly different.

	Caecal V.F.A. Conc ⁿ mg.g ⁻¹ n = 9	Caecal V.F.A. Content mg n = 9	Faecal V.F.A. Conc ⁿ mg.g ⁻¹ n = 9	Faecal V.F.A. Output mg.24h ⁻¹ n = 9
Elem-D	26.4 ± 6.3 ^a	13.0 ± 3.4 ^b	4.4 ± 1.2 ^c	5.0 ± 2.5
Elem-D + 13% G.A.	13.7 ± 7.7 ^a	24.4 ± 9.5 ^b	8.6 ± 2.7 ^c	5.4 ± 1.8

a p < 0.001
b p < 0.01
c p < 0.001

TABLE 6

Individual V.F.A. concentrations as a proportion of total V.F.A. concentration in the caecal content and faeces of rats fed Elem-D or Elem-D + 13% G.A. (mean % ± S.D.). Figures with common superscripts are significantly different.

	<u>Caecal Content</u>		<u>Faeces</u>	
	Elem-D	Elem-D + G.A.	Elem-D	Elem-D + G.A.
Acetate	54.6 ^a ±3.0	59.6 ±13.7	65.1 ^a ±8.1	65.3 ±3.0
Propionate	18.1 ±0.9	20.4 ±5.5	16.6 ±1.8	18.9 ±2.7
Butyrate	13.8 ^b ±2.2	17.1 ±6.1	9.4 ^{b,c} ±3.8	14.8 ^c ±4.1
Acetate: Propionate	3.0	2.9	4.1	3.5
Acetate: Butyrate	4.0	3.5	6.9	4.4
Propionate: Butyrate	1.3	1.2	1.8	1.3

a p < 0.005
b p < 0.01
c p < 0.01

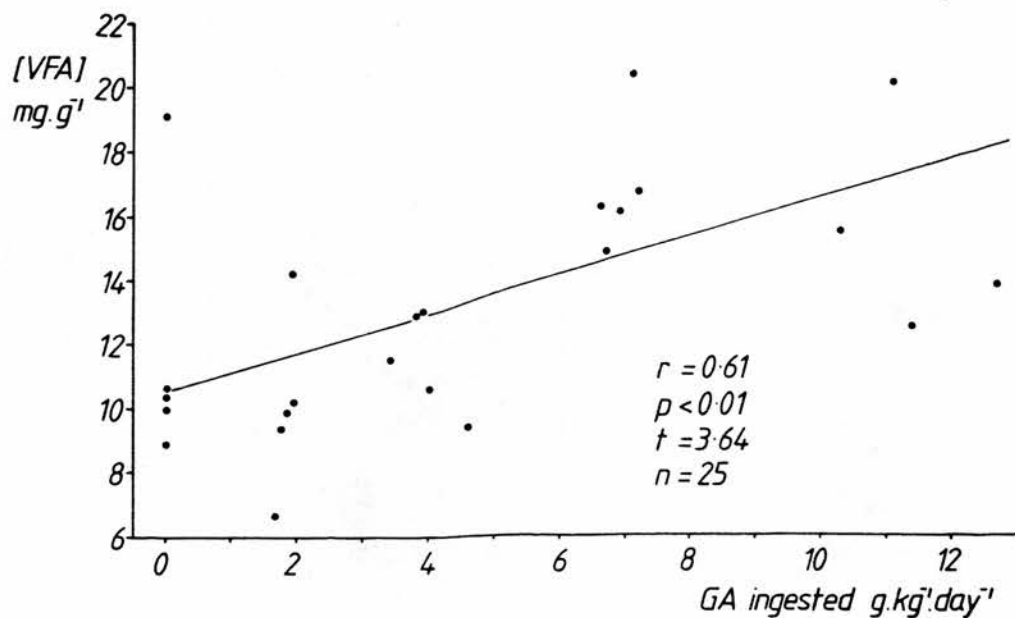


Fig.1: V.F.A. concentrations (mg.g⁻¹) in dried faeces of rats fed various doses (g.kg⁻¹.24h⁻¹) of G.A. in a pellet diet (O.B.D. + G.A.)

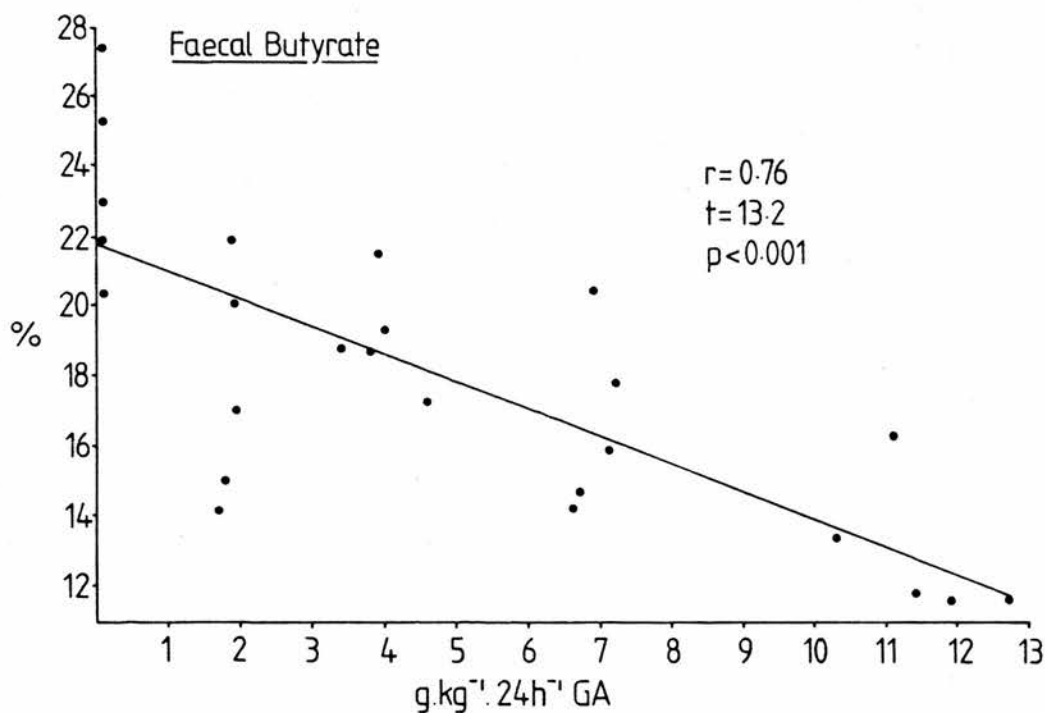


Fig.2A

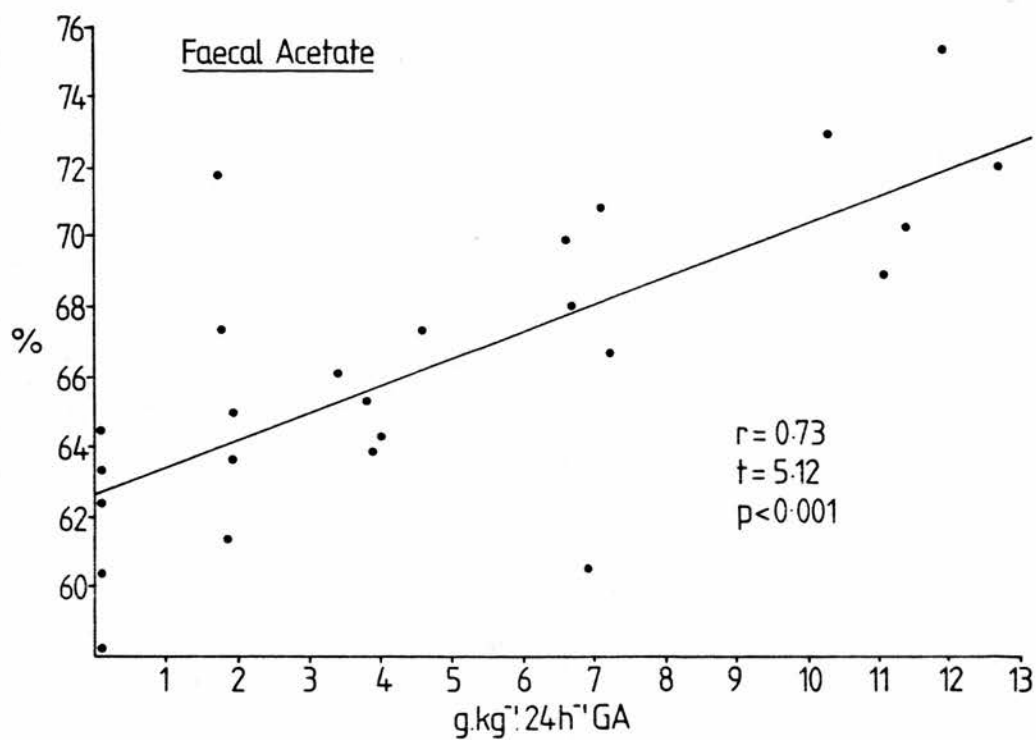


Fig.2B

A/B: Butyrate and acetate concentration as percentage of total faecal V.F.A. concentration in faeces of rats fed various amounts of O.B.D. + G.A.

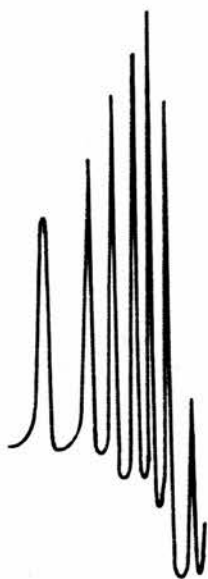


Fig.3: G.L.C. elution patterns of the 6 naturally occurring V.F.A.s. Peaks left to right: internal standard, valerate, isovalerate, butyrate, isobutyrate, propionate, acetate.

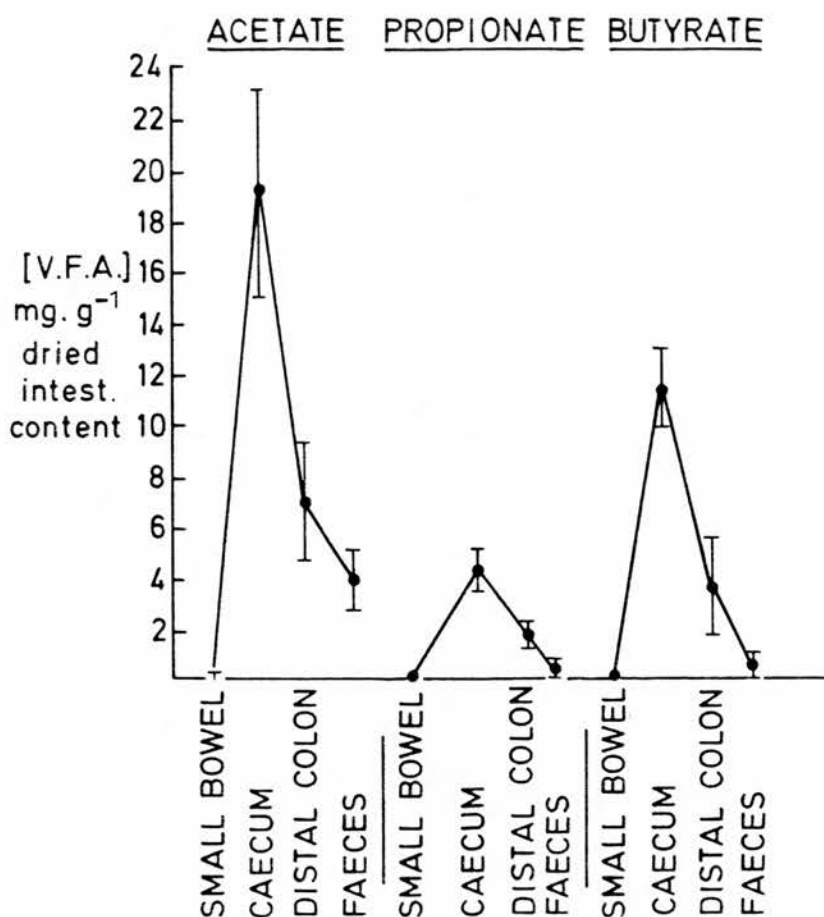


Fig.4: V.F.A. concentrations (mg.g⁻¹) at various points in the intestine of rats (n=5) fed a Spratts pellet diet ad libitum (mean \pm S.D.).

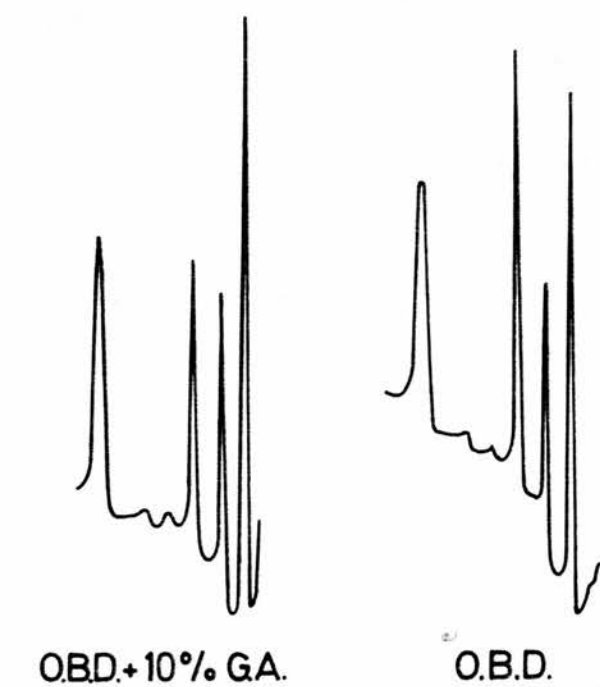


Fig.5: G.L.C. elution pattern of V.F.A.s in the faeces of rats fed O.B.D. or O.B.D. + G.A.

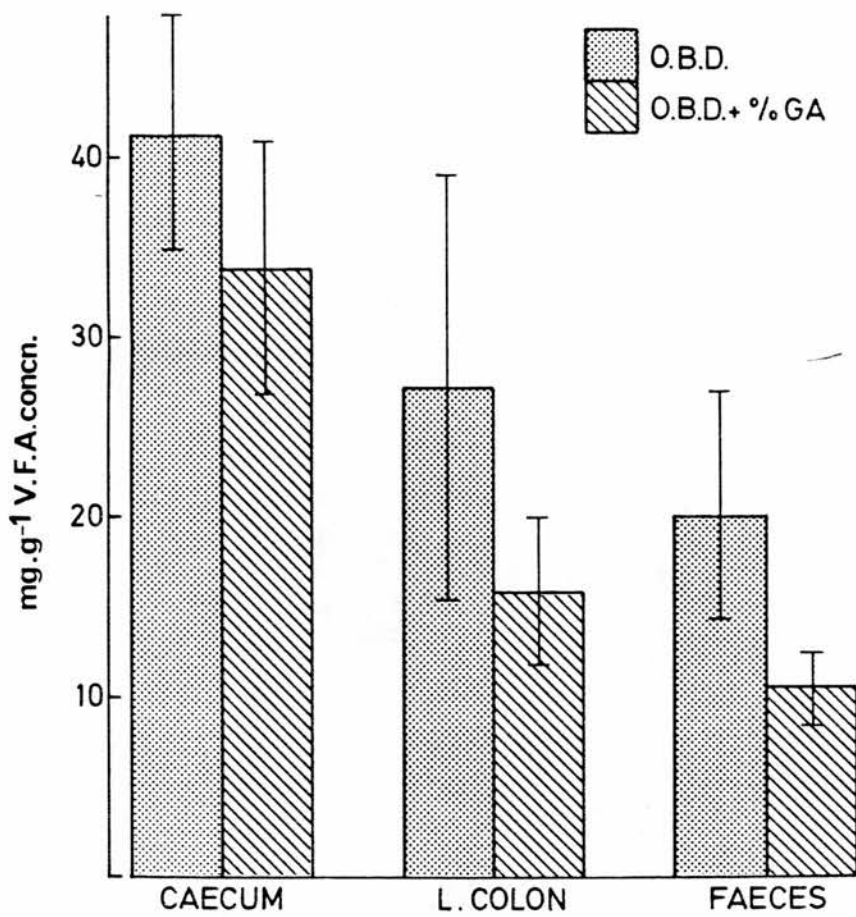


Fig.6: Faecal, colonic and caecal V.F.A. concentrations (mg.g^{-1}) in rats fed O.B.D. or O.B.D. + 10% G.A. (mean \pm S.D.: n=5 for each group).

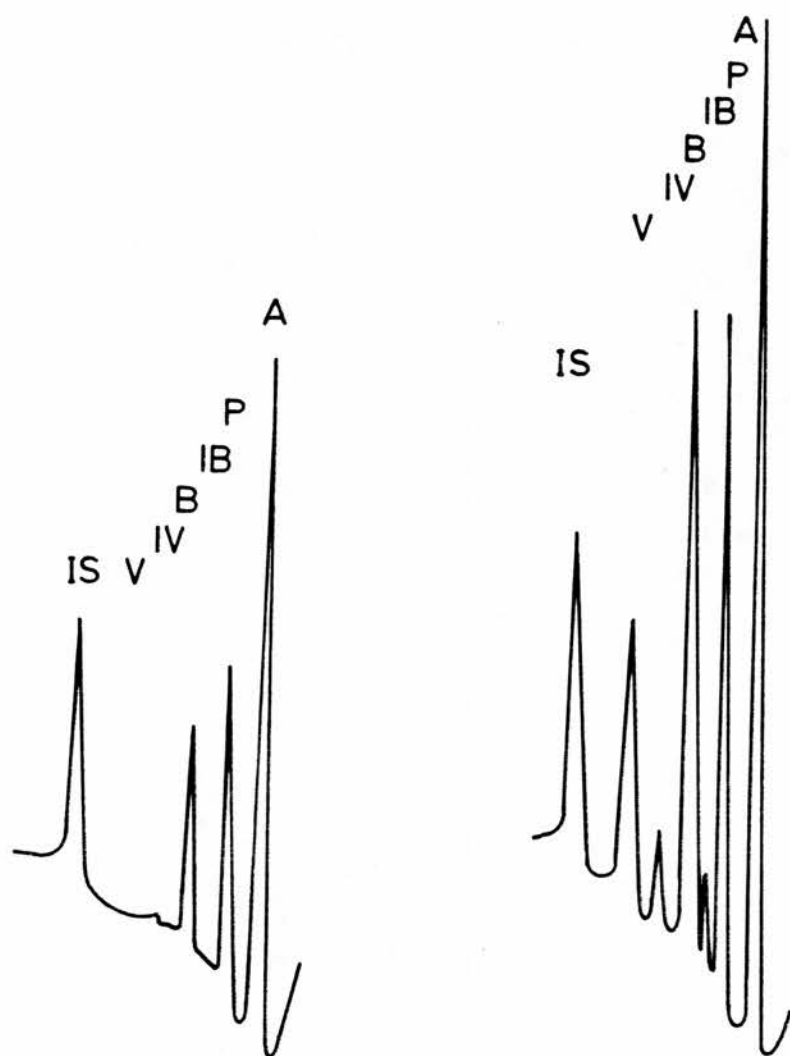


Fig.7: G.L.C. elution pattern of V.F.A.s in caecal content of rats fed Elem-D (right) or Elem-D + 13% G.A. (left).

Intestinal gas production during G.A. administration to rats.

Albino Wistar rats, 3 months old, were fed a pellet diet (O.B.D. or O.B.D. + 20% G.A.) ad libitum, or elemental diet (Elem-D or Elem-D + 13% G.A.) ad libitum, for 28 days. 3 month old rats were chosen since at this age they universally produce both methane and hydrogen. Samples of exhaled gas were collected at days 0, 14 and 28 by the method described previously (pp 50). Samples were obtained over 15 minute periods and were collected between 0900 and 1100 hrs. after ad libitum feeding overnight.

3 albino Wistar rats, previously subjected to caecal excision, and allowed to recover for 2 months were given O.B.D. + 20% G.A. for 4 weeks. Breath hydrogen and methane excretion were measured as in the previous experiment.

Results

Rats on O.B.D. diet showed a significant reduction in hydrogen excretion at 28 days ($p < 0.002$) compared to control values. Methane excretion remained unaltered. (Table 1).

The O.B.D. + 20% group similarly showed a significant reduction in hydrogen excretion at 28 days. Methane production rose significantly at 28 days ($p < 0.001$).

In contrast hydrogen and methane production could not be detected at either 14 or 28 days in the group consuming Elem-D. In the Elem-D + 13% G.A. group methane and hydrogen were undetectable at 14 days but reappeared at 28 days at levels insignificantly different from pretreatment values.

Rats that had previously undergone caecal excision excreted no methane on either O.B.D. or O.B.D. + 20% G.A. One rat excreted hydrogen and was subsequently found at p.m. to have evidence of subacute small intestinal obstruction at the site of the anastomosis.

TABLE 1

methane and hydrogen excretion ($\text{ml.kg}^{-1}.\text{h}^{-1}$) from rats fed O.B.D., O.B.D. + 20% G.A., Elem-D, Elem-D + 13% G.A. Mean \pm S.D. (n.d. = not detected). Figures with common superscripts are significantly different.

		<u>Methane</u>		
		DAY 0	DAY 14	DAY 28
O.B.D.	n = 5	0.25 ± 0.22	0.10 ± 0.01	0.15 ± 0.05
O.B.D. + 20% G.A.	n = 5	0.33 ± 0.14^a	0.95 ± 0.46	0.98 ± 0.24^a
Elem-D	n = 3	0.53 ± 0.33	n.d.	n.d.
Elem-D + 13% G.A.	n = 3	0.65 ± 0.32	n.d.	0.25 ± 0.06
		<u>Hydrogen</u>		
O.B.D.	n = 5	0.69 ± 0.13^b	0.29 ± 0.23	0.36 ± 0.07^b
O.B.D. + 20% G.A.	n = 5	0.86 ± 0.28^c	0.82 ± 0.58	0.42 ± 0.15^c
Elem-D	n = 3	0.21 ± 0.17	n.d.	n.d.
Elem-D + 13% G.A.	n = 3	0.67 ± 0.25	n.d.	0.98 ± 0.36

a $p < 0.001$

b $p < 0.002$

c $p < 0.02$

Chapter 3 (viii)

Small bowel morphology and cell kinetics in rats fed gum arabic

Adult male Liverpool Hooded rats were fed O.B.D. or O.B.D. + G.A. for 2 years. Diets were available ad libitum. Five dietary regimens were employed: O.B.D., O.B.D. + 2.5% G.A., O.B.D. + 5.0% G.A., O.B.D. + 10.00% G.A., O.B.D. + 20.00% G.A.

Three rats were used from each dietary group. Rats were administered vincristine sulphate (Oncovin) (1 mg.kg^{-1}) by intraperitoneal injection at the start of the experiment (0 hrs.). One animal from each treatment group was sacrificed by cervical dislocation at 30, 60 and 90 minutes from the time of vincristine administration. After evisceration a 2 cm. length of mid jejunum was removed from each animal, opened and placed in Clarke's fixative for 24 hours before being transferred to 75% ethanol for storage.

Later, the jejunal segments were stained, and examined for crypt depth, villus height and number of cells arrested in metaphase per crypt as previously described in Chapter 2 pp 54-55.

Results

Jejunal villus height and crypt depth measurements are noted in Table 1. One rat in the O.B.D. + 2.5% G.A. dietary group demonstrated partial villus atrophy (villus height 28.3μ). No morphological abnormalities were detected in the other 14 animals. Mean crypt depth and villus height were similar in all treated groups when compared to controls.

Jejunal cell kinetic studies

The relationship between crypt cell production rate and the ingested dose of gum arabic is noted in Figure 1. A relationship could be shown ($r = 0.834$) wherein crypt cell production rate was significantly decreased in the O.B.D. + 20% G.A. group of rats ($p < 0.005$) Fig. 2. No significant differences exist between controls and the 2.5%, 5.0% and 10.0% dietary groups.

TABLE 1

Jejunal crypt depth and villus height in rats fed O.B.D. or O.B.D. + G.A. for two years (mean \pm S.D.).

		G.A. Dose (%)	0	2.5	5.0	10.0	20.0
Mean Crypt Depth. μ	n = 3		194.0 \pm 10.9	184.5 \pm 9.8	188.2 \pm 4.5	199.9 \pm 14.3	187.0 \pm 18.8
Mean Villus Height. μ	n = 3		764.4 \pm 17.4	727.3 \pm 65.0	728.5 \pm 65.0	741.2 \pm 80.0	759.8 \pm 67.3

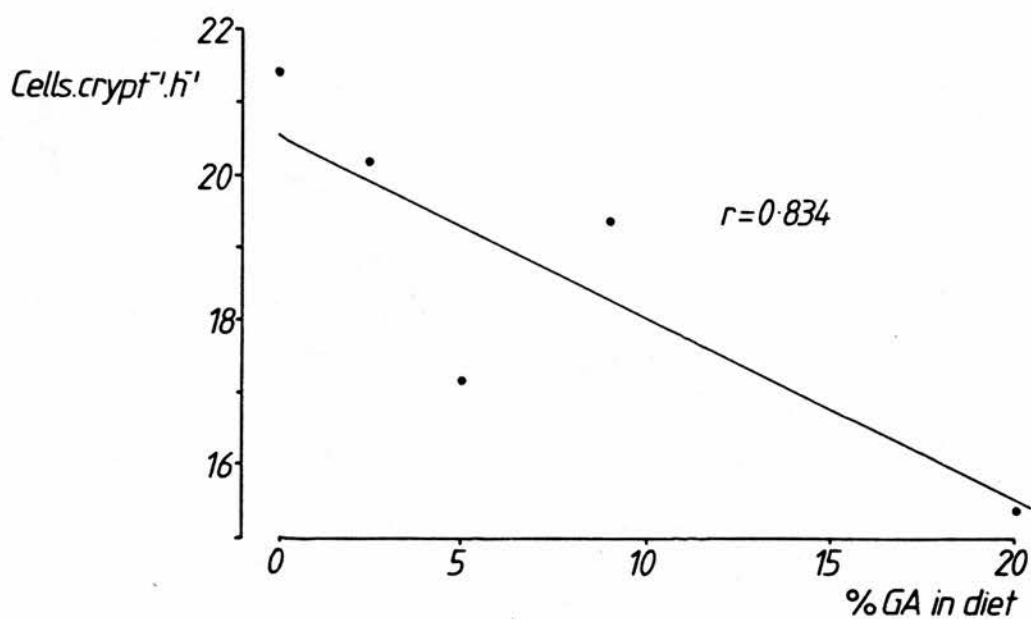


Fig.1: Jejunal crypt cell production rate (cells.crypt⁻¹.h⁻¹) in rats fed O.B.D. or O.B.D. + G.A. for 2 years.

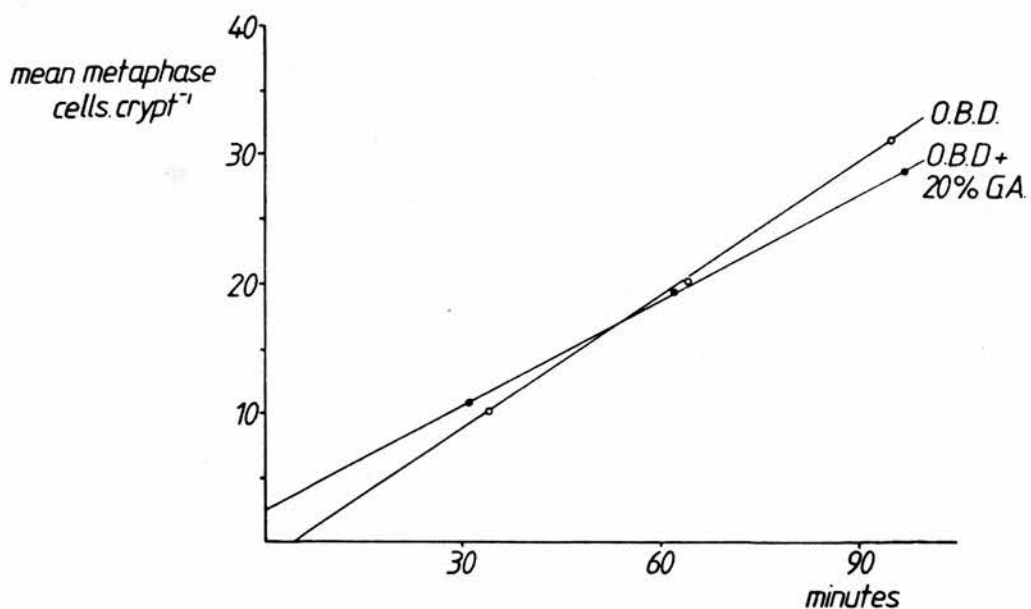


Fig.2: Jejunal crypt cell production (mean metaphase cells.crypt⁻¹) in animals fed O.B.D. or O.B.D. + 20% G.A.

In vitro studies of the digestion of gum arabic

In an attempt to determine the effect of the endogenous secretions of the human upper gastrointestinal tract on gum arabic, in vivo incubation experiments were carried out using human gastric and pancreatic juice.

Materials: Human gastric juice was obtained from a patient who underwent a maximal acid output estimation following pentagastrin administration. Human pancreatic juice was obtained from a patient with a pancreaticocutaneous fistula.

Methods

G.A. (2g) was incubated with human gastric or pancreatic juice (40ml) for 72 hours. During incubation the temperature was maintained constant (37°C) by a heated water bath. The solutions were gently agitated throughout the experiment. A control solution was made up of gastric juice to which G.A. had not been added. Aliquots (2ml) were removed at intervals (0, 1/2, 1, 2, 3, 4, 6 and 72 hours. G.A. was precipitated from solution by addition of acidified ethanol (95%). Precipitates obtained were spun down, dried overnight in an oven (50°C) and then accurately weighed. The supernatants were concentrated by evaporation and examined for sugars using thin layer chromatography. The precipitates were hydrolysed and the hydrolysate examined using paper or thin layer chromatography.

Results

White flocculent precipitates typical of G.A. were obtained from the solutions to which G.A. had been added but not from the control solution. The dried weights of the precipitants are recorded in Table 1. Each 2ml. aliquot of digesta would be expected to contain 100mg G.A. Small and inconstant variations from this value are noted up to 72 hours. Excess weights where present were thought to be at least partly derived from the

precipitation of substances such as mucoproteins found in the gastric and pancreatic juices. The pancreatic digesta appeared most prominently to produce this excess though the quantities were never large (± 22 mg) from either solution. There appeared to be no decrease in weight of precipitate to suggest progressive breakdown of G.A. which appeared to be substantially intact judged by recovery weights at the conclusion of the experiment.

Supernatant chromatography

Chromatographic examination of the concentrated supernatants demonstrated the absence of identifiable monomers of G.A. Some very indistinct sugar spots were occasionally identified and one of the most constant of these was thought to correspond with fucose.

Precipitant Hydrolysate Chromatography

Hydrolysis of precipitates from the control solution was impractical due to their small size. Chromatography of the hydrolysates of precipitates from the G.A. containing solutions revealed sugar migrations characteristic of the gum, i.e containing rhamnose, galactose and arabinose.

TABLE 1

weights (mg.) of dried precipitate obtained following addition of acidic ethanol
to gastric juice and pancreatic juice containing G.A.

	(hB)	0	$\frac{1}{2}$	1	2	3	4	6	72
CONTROL (m.g.)		0	1.6	0.1	1.1	1.1	2.3	1.5	0.7
gastric juice G.A. (m.g.)		95.3	103.6	109.8	104.0	102.8	121.3	100.6	97.9
pancreatic juice G.A. (m.g.)		103.9	102.6	106.8	105.8	106.3	115.5	101.7	110.4

Chapter 3 (x)

In vivo studies of G.A. in man

Materials and Methods

Five male volunteers aged between 30 - 55 years and free from known gastrointestinal disease were selected for study. The design of the study which lasted for four weeks is summarised in Fig.1. Subjects consumed their normal diet throughout the study, and were instructed not to take laxatives or antibiotics during the four week period. Only one subject was a habitual smoker. Smoking was not allowed on days when breath gas excretion was studied. In the first week a five day faecal collection was made and during this time diet diaries, recording the nature and quantity of food consumed, were kept by each participant. On the first day of faecal collection 40 radio-opaque plastic markers were swallowed at 09.00 hrs. In each of the final 21 days of the study, gum arabic solution (124 ml. 25% syrup) were taken prior to breakfast. Compliance was ensured by daily contact with the subjects and by bottle counts. A further faecal collection was taken over the last five days of the study. Diet diaries were again kept during this period. The first and 28th days were designated study days when a number of investigations were carried out. These were: serum biochemistry (SMAC analysis), extended glucose tolerance test (G.T.T.) after an oral glucose load (50g), plasma insulin, breath hydrogen and methane excretion during the oral G.T.T., and blood haematology. All subjects fasted from midnight on the night prior to the study day, though G.A. was taken at the usual time immediately prior to the G.T.T.

Other studies, separate from this central study were carried out and these are referred to where appropriate.

Results

No significant alterations in diet occurred during the two periods of study. In particular cholesterol and fibre

intake (excluding gum arabic) were insignificantly different. Table 1. All participants complied with the study design and G.A. was consumed appropriately as judged by bottle counts and interview.

Three of the five subjects noted abdominal distension and occasional colic while taking G.A. All subjects noted the passage of increased quantities of flatus per rectum.

Serum biochemistry

Most of the biochemical measurements were unchanged. There were small reductions in the serum alanine aminotransferase aspartate aminotransferase activities in all subjects ($p < 0.0025$, $p < 0.001$ respectively) and though these activities remained within the limits of a normal population for A.S.T. the post gum mean level of A.L.T. was slightly below the low normal limit for the laboratory. No other significant alterations occurred (Table 2).

Fig. 2 demonstrates the effect of G.A. on fasting serum lipids. Serum triglycerides and phospholipid concentrations were unaltered by G.A. ingestion. Total serum cholesterol concentration was reduced in all subjects after consumption of G.A., the mean decrease was 0.39 mmol.l^{-1} .

The addition of G.A. to an oral G.T.T. had no significant effect on post prandial glucose concentration at any time and this was mirrored by an absence of any significant change in plasma insulin concentrations. Figs. 3 & 4.

No significant alterations in haematological parameters occurred. (Table 3).

Faecal characteristics of constituents

Completeness of collection was confirmed by the recovery

of pellet markers which was greater than 90% in all subjects. Subjects demonstrated variable responses to ingestion of G.A. but overall no significant alterations in the faecal constituents of characteristics measured occurred (Figs. 5 & 6).

Correlations between wet and dry stool weight and transit time with faecal constituents are noted in Table 4. Wet stool weight correlated significantly with daily V.F.A. and bile acid excretion ($p < 0.001$, $p < 0.05$). Dry stool weight correlated with daily excretion of faecal fat bile acids and V.F.A. ($p < 0.05$, $p < 0.05$, $p < 0.01$ respectively). Transit time was correlated with none of the measured faecal constituents.

Attempts were made to recover G.A. from the faeces of subjects taking G.A. (25gd^{-1}). In order first to establish whether G.A. present in human faeces could be separated out a recovery experiment was designed. Purified G.A. was added to a faecal slurry to achieve a final G.A. concentration of 10%. After thorough mixing the slurry was subjected to bench and high speed centrifugation. The supernatant obtained was added to acidified ethanol (95%) (1:4 v/v). A white flocculent precipitate typical of G.A. was obtained.

Stool slurry was obtained from the individually pooled faecal collections of 2 subjects before and after consumption of G.A. for 3 weeks. Samples were diluted (1:1 v/v) in distilled water and after thorough mixing, centrifuged as before. The supernatant obtained was added (1:4 v/v) to acidified ethanol (95%). A similar meagre, brown, gelatinous precipitate was obtained from both pre and post gum faecal slurries. Fig.7. Hydrolysis and chromatography of this precipitate was not attempted because of: 1. the similarity of the precipitates in pre and post gum samples, and 2. the previous lack of success in attempts to perform this on supernatant precipitates from the rat intestine.

Breath hydrogen and methane excretion

Breath hydrogen excretion measured during the extended G.T.T. before and after G.A. ingestion is noted in Fig.8. During the G.T.T. unsupplemented by G.A., hydrogen concentrations remained low throughout declining as the test proceeded as is characteristic in fasting individuals. During the G.A. supplemented G.T.T. breath hydrogen concentrations remained unaltered up to 120 minutes. Thereafter significant increases in breath hydrogen concentrations were seen at 120, 150, 180, 210 and 240 minutes ($p < 0.05$, $p < 0.02$, $p < 0.025$, $p < 0.01$, $p < 0.05$ respectively).

As, in theory, it was possible that the G.A. solution was simply carrying glucose unabsorbed into the caecum where its fermentation resulted in hydrogen production, 2 of the 5 subjects were restudied. G.A. (25g) without glucose flavouring or supplements was administered to the two subjects who were fasted. Breath hydrogen excretion over the ensuing 6 hours is seen in Fig.9. Both subjects demonstrated increases in breath hydrogen commencing at 120 minutes after consumption of G.A. indicating G.A. was the subject of fermentation.

Since both these experiments reflected a response to the chronic ingestion of large quantities of G.A. a further experiment to assess the effects of G.A. on individuals with no previous exposure to high doses of G.A. was designed. Five separate male volunteers, apparently free of significant alimentary disease, were fasted overnight. G.A. syrup (125 mls 20% syrup) was administered at 0900 hours the following morning and breath gas excretion monitored for five hours. Two subjects were found to have relatively high levels of fasting breath hydrogen (16 & 18 p.p.m.) and results have therefore been expressed as mean deviations (p.p.m.) from initial concentrations. One of these two subjects demonstrated marked peaks and troughs of breath H_2 excretion throughout the experiment but overall there was no response to G.A. ingestion in these subjects (Fig.10). None of the other four subjects showed a rise in breath hydrogen greater than 4 p.p.m.

after acute G.A. ingestion.

Methane excretion

Three of the subjects produced methane at the commencement of the study of chronic ingestion of G.A. At the end of this two of these subjects had undetectable quantities of methane in exhaled breath and the third demonstrated unaltered methane excretion. Fig.11. In the acute ingestion study methane was detectable in low levels in four subjects (2-3 p.p.m.) and remained unaltered after ingestion of G.A.

TABLE 1

Dietary intake during control and final week of study. (Median and range).

<u>Dietary Intake</u>		
	Control period	After 3 weeks gum arabic
Protein (g)	88 (76 - 132)	98 (80 - 130)
Fat (g)	111 (95 - 180)	122 (86 - 176)
Carbohydrate (g)	304 (178 - 407)	253 (136 - 370)
Calories	2672 (2148-3884)	2620 (1992 - 3627)
Sugar (g)	113 (31 - 169)	94 (30 - 122)
Alcohol (g)	34 (18 - 38)	41 (6 - 51)
Fibre (g)	23 (11.5 - 38)	20 (11 - 21)
Cholesterol (g)	0.73 (0.25 - 1.2)	0.68 (0.3 - 1.06)

Serum biochemical indices in male subjects before and after the ingestion of G.A. for three weeks (mean \pm S.D.).

	Urea	Na ⁺	K ⁺	CO ₂	Bili- rubin	ALT	ALP	Ca ⁺⁺	PO ₄ ⁼⁼	Creat.	Uric	AST	Prot.	Alb.
Normal	2.5-6.6	132-144	3.3-4.7	24-30	2-17	10-40	40-100	2.12-2.62	0.8-1.4	55-150	0.12-0.42	10-35	60-80	36-147
Range	mmol.l ⁻¹	mmol.l ⁻¹	mmol.l ⁻¹	mmol.l ⁻¹	μ mol.l ⁻¹	units.l ⁻¹	units.l ⁻¹	mmol.l ⁻¹	mmol.l ⁻¹	μ mol.l ⁻¹	μ mol.l ⁻¹	units.l ⁻¹	g.l ⁻¹	g.l ⁻¹
Pre-gum (mean)	4.96	140	3.9	27	12	15	60	2.35	0.97	90	0.36	20.4	69	43
Post- gum (mean)	4.82	140	4.0	28	10	7 ^a	65	2.36	0.96	94	0.37	15 ^b	72	44
Mean difference \pm S.D.	-0.14 + 1.13	0.4 + 2.19	0.1 + 0.24	0.8 + 2.68	-2.2 + 2.59	-8.0 + 2.35	5.4 + 6.8	0.006 + 0.06	-0.008 + 0.15	3.8 + 8.79	0.004 + 0.04	-5.6 + 1.14	2.8 + 2.78	1.0 + 2.73

a p < 0.0025

b p < 0.001

Haematological indices in male subjects before and after the ingestion of G.A. for 3 weeks. (Mean \pm S.D.).

	WBC $\times 10^9/l$	RBC $\times 10^{12}/l$	Hb g/dl	Hct	MCV fl	MCH pg	MCHC g/dl	Platelets $\times 10^9/l$	Retics $\times 10^9/l$
Pre-gum (mean)	6.28 ± 0.59	5.22 ± 0.47	16.14 ± 1.1	0.476 ± 0.03	91 ± 4.7	30.7 ± 1.65	33.4 ± 0.21	19.3 ± 26.2	64.2 ± 41.1
Post-gum (mean)	6.72 ± 0.76	5.21 ± 0.16	15.9 ± 0.9	0.474 ± 0.02	90.4 ± 4.4	30.3 ± 1.46	33.3 ± 0.38	18.6 ± 0.38	28.6 ± 25.4

TABLE 4

Correlations between faecal characteristics and constituents.

			r
Wet stool weight g/24h	v.	transit time	-0.35
		faecal : fat/24h	0.47
		total bile acids/24h	0.72 p < 0.05
		total volatile fatty acids/24h	0.96 p < 0.001
		acetate/24h	0.57
		propionate/24h	0.52
		butyrate/24h	0.67 p < 0.05
Dry stool weight	v.	faecal : fat/24h	0.73 p < 0.05
		volatile fatty acids/24h	0.81 p < 0.01
		bile acids/24h	0.71 p < 0.05
Transit time	v.	faecal : fat/24h	0.40
		total volatile fatty acids/24h	-0.43
		total bile acids/24h	-0.18

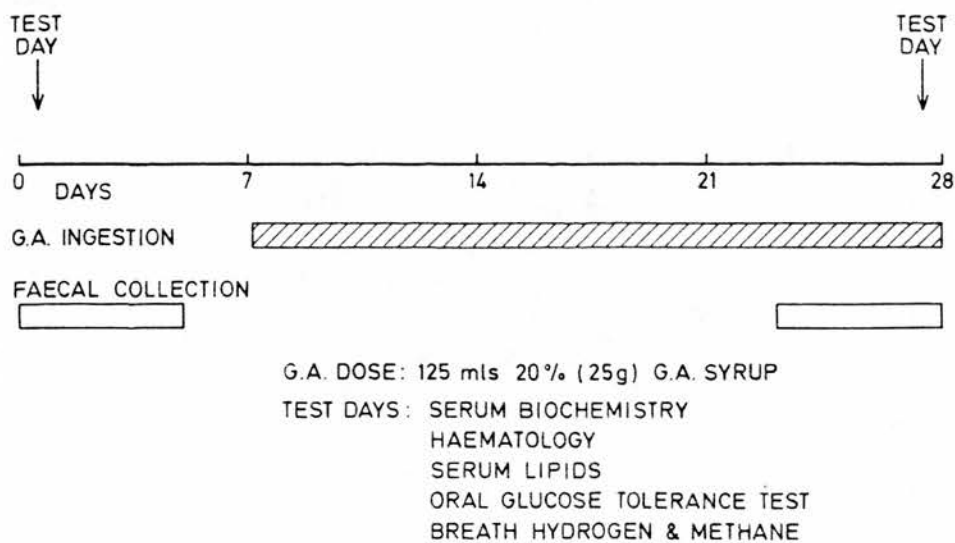


Fig.1: Diagrammatic representation of the human study design.

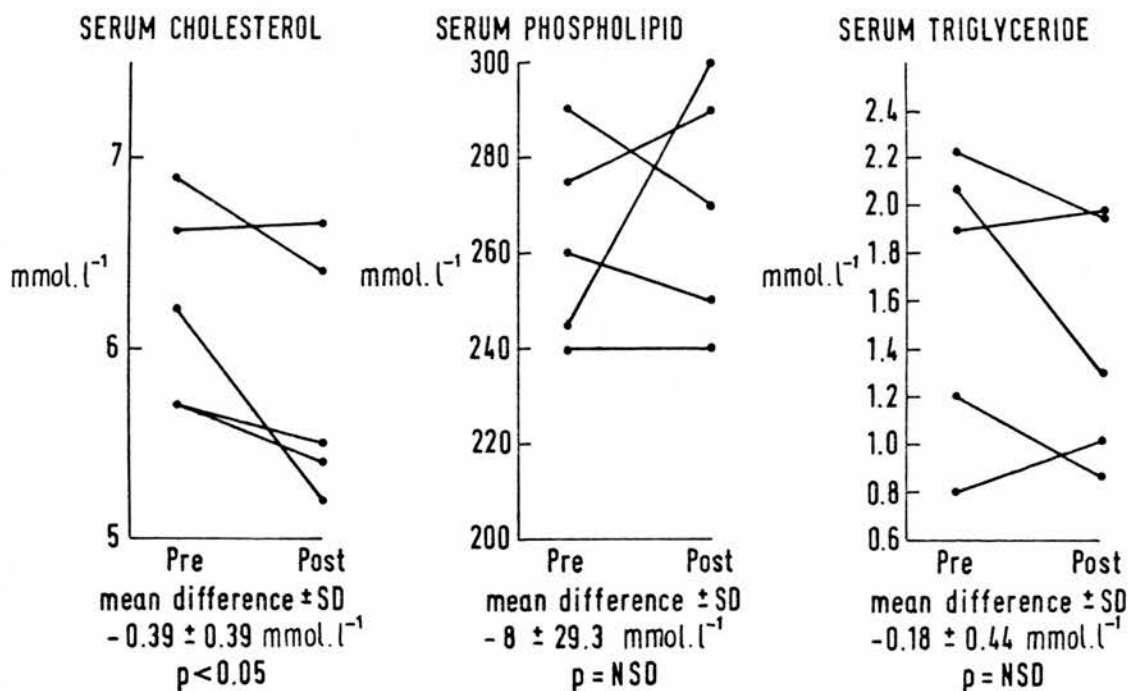


Fig.2: Changes in serum lipids in male subjects before and after ingestion of G.A. for 3 weeks. Mean differences \pm S.D. between control and 3 week values are noted beneath.

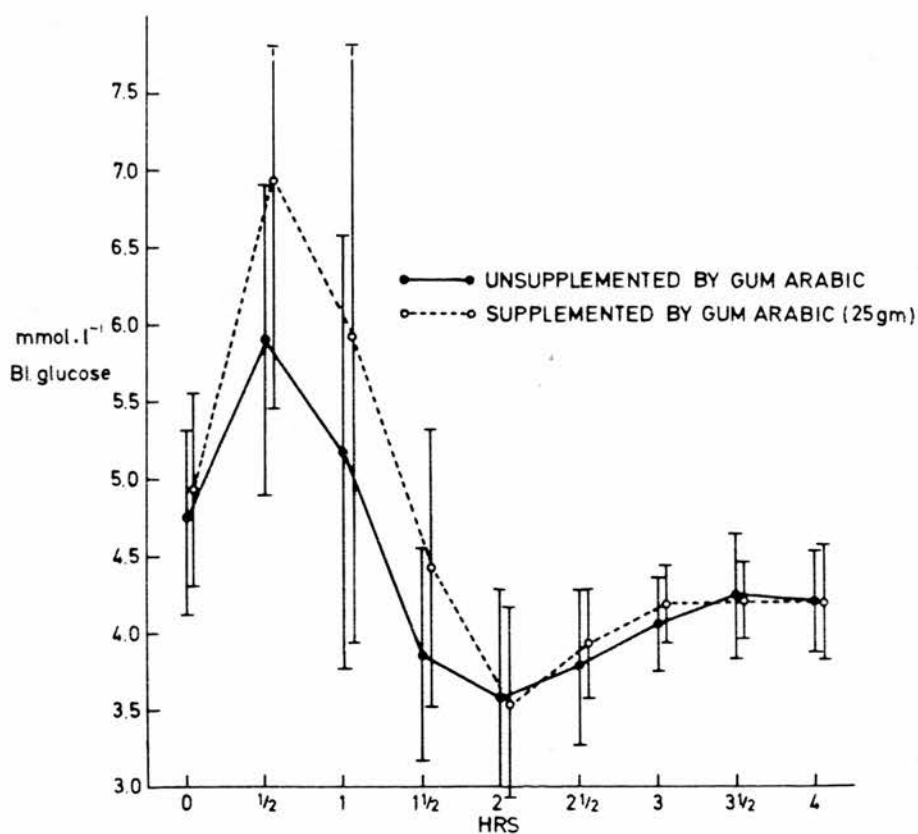
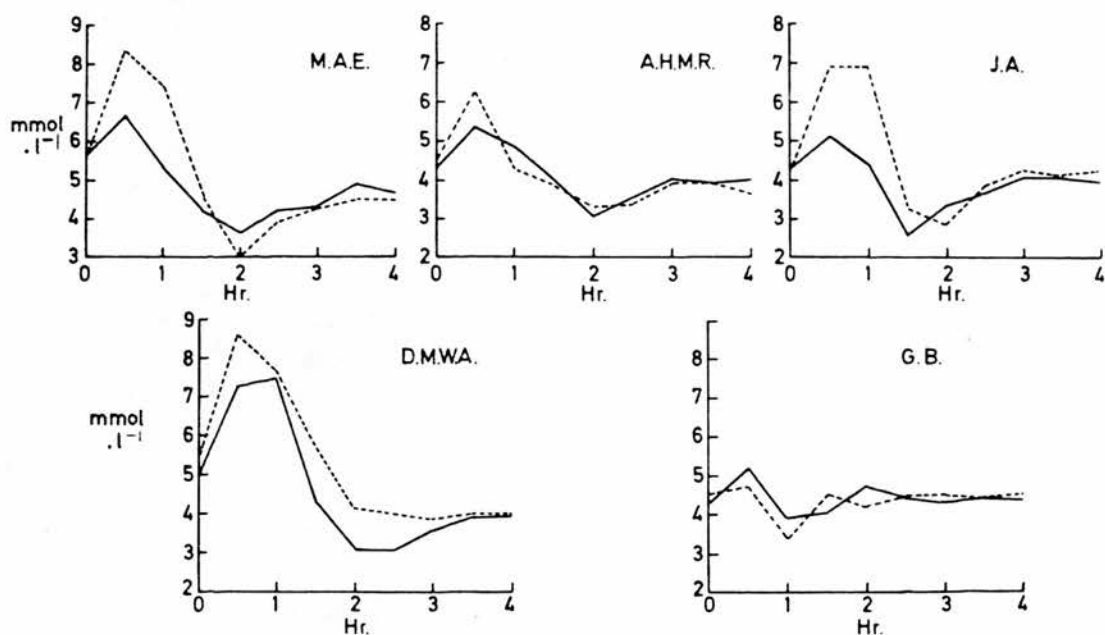


Fig.3: Blood glucose responses to the ingestion of glucose (50g) by male subjects (n=5) before (solid lines) and after (hatched lines) taking G.A. (25g). Individual G.T.T. Fig.A, mean G.T.T. Fig.B. (\pm S.D.)

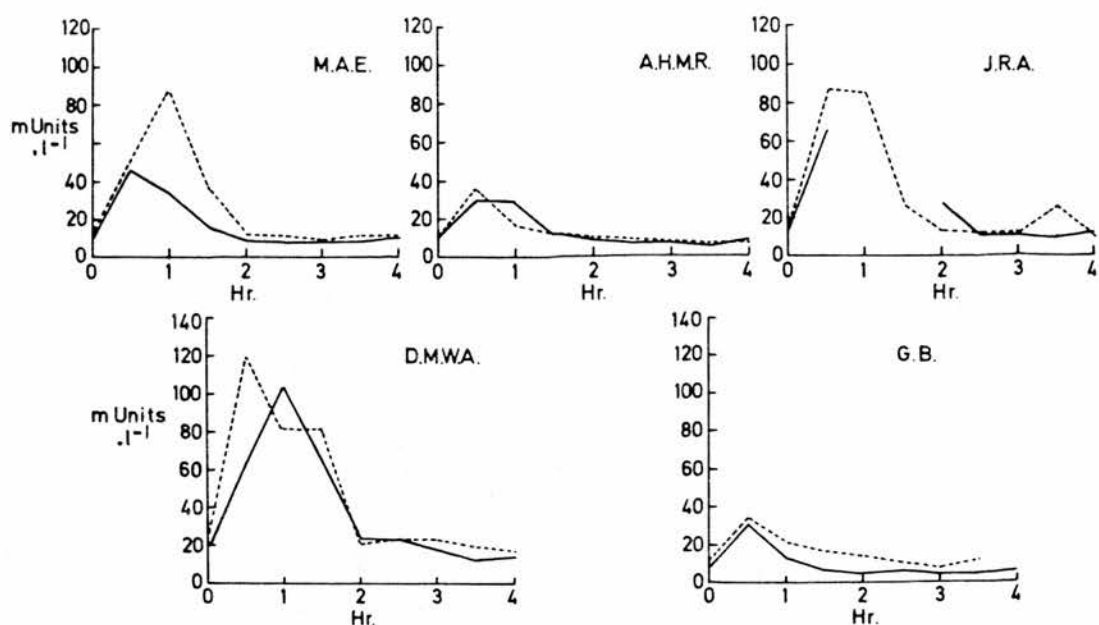


Fig.4: Plasma insulin responses to the ingestion of glucose (50g) by male subjects (n=5) before and after taking G.A. (25g).

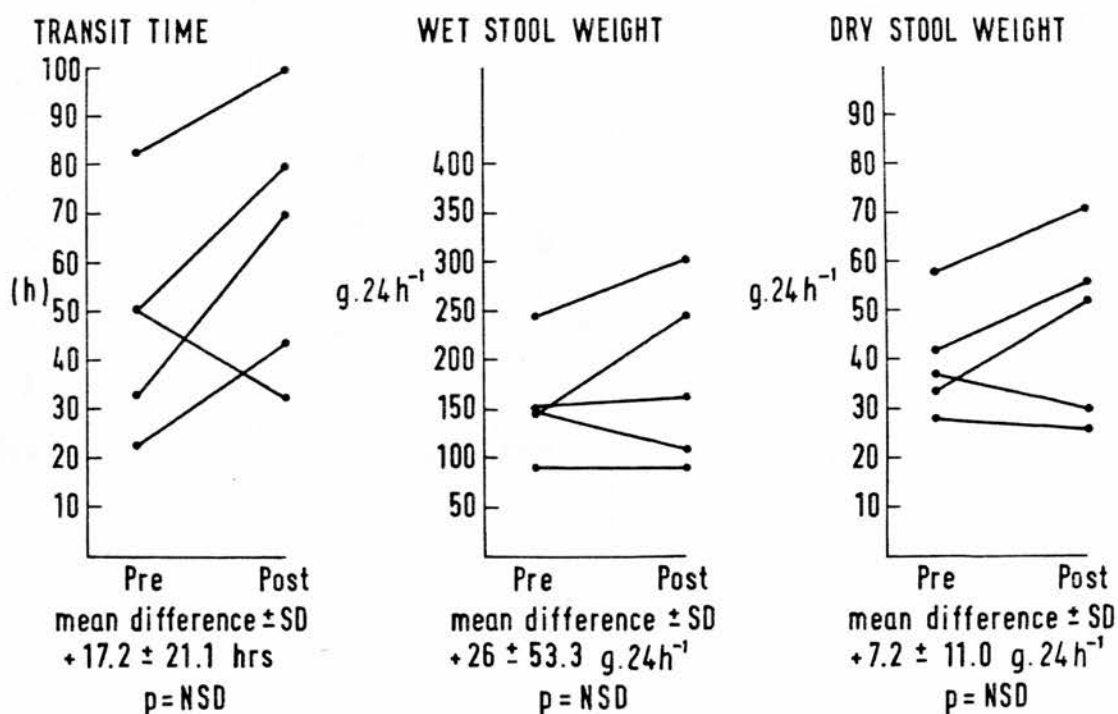


Fig.5: Alterations in faecal characteristics in subjects before and after taking G.A. ($25.\text{g.d}^{-1}$) for 3 weeks.

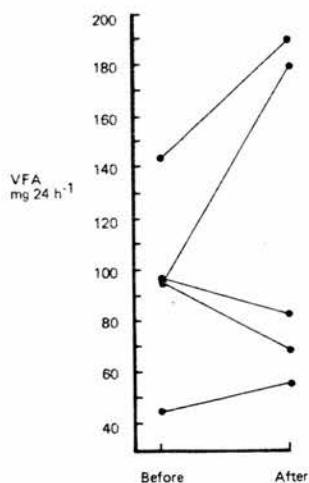
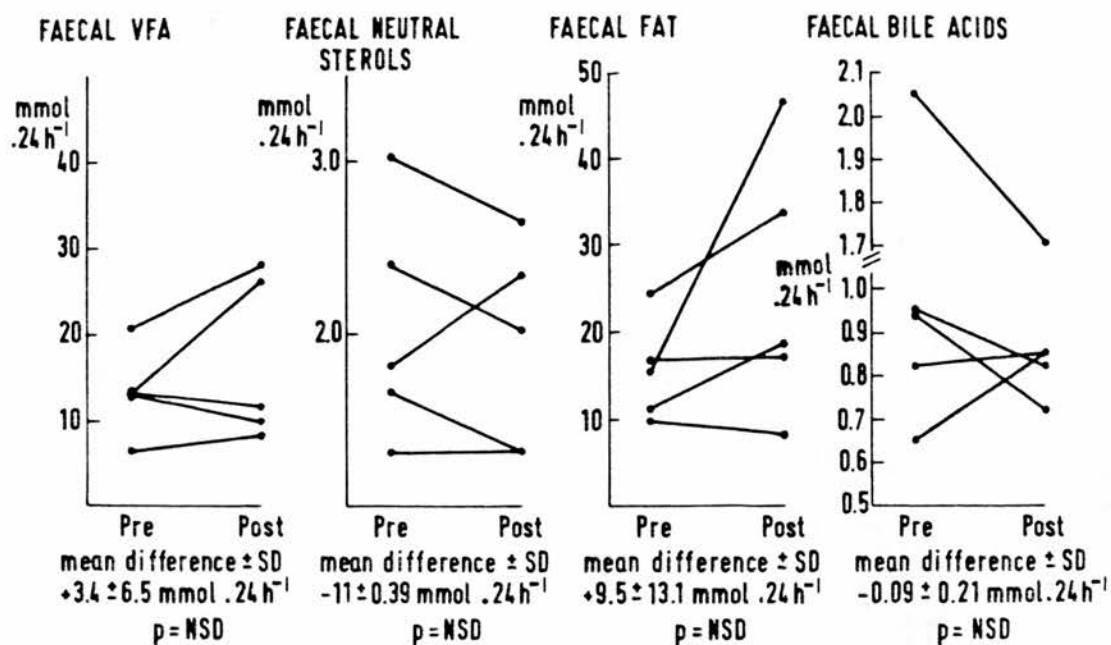


Fig.6: Alterations in faecal biochemistry in subjects before and after taking G.A. (25g.d⁻¹) for 3 weeks. (Values for V.F.A. are separately quoted in mmol. and mg.).



Fig.7: The results of acidified ethanolic precipitation of supernatants from centrifuged stool slurry from subjects taking a control (left) and a gum supplemented (right) diet.

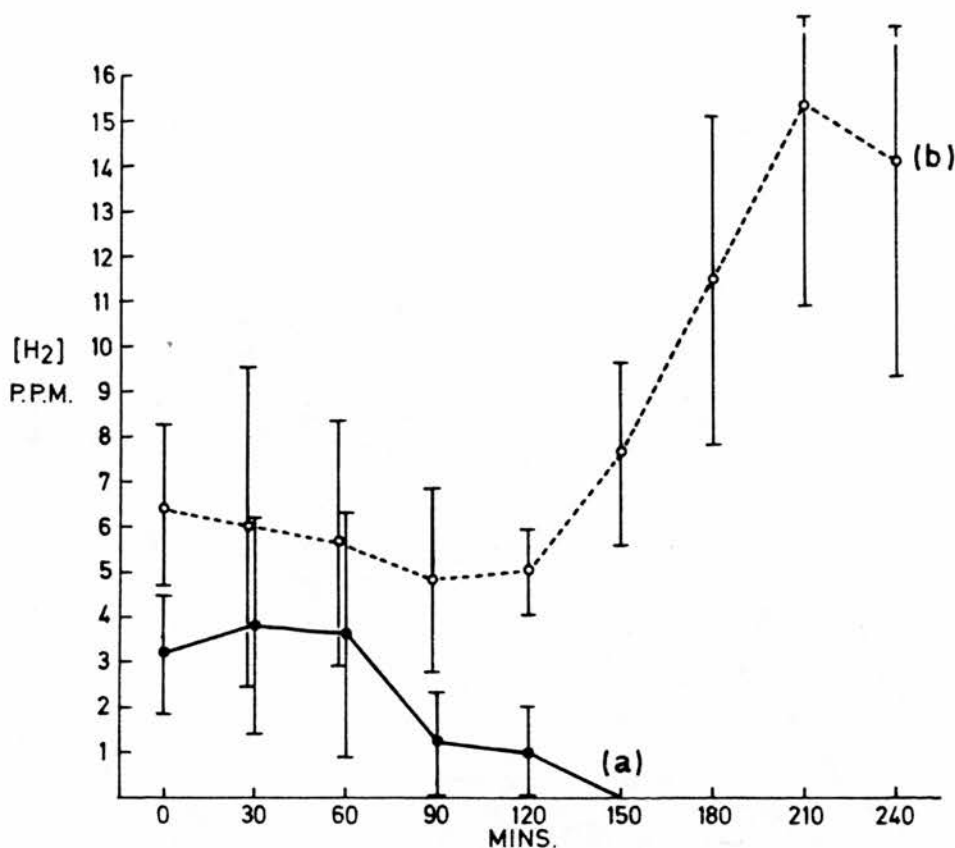


Fig.8: Breath hydrogen concentrations in male subjects (n=5) before and after taking G.A. (25g.d⁻¹) for 3 weeks. (p.p.m. mean \pm S.D.). a before G.A., b after G.A.

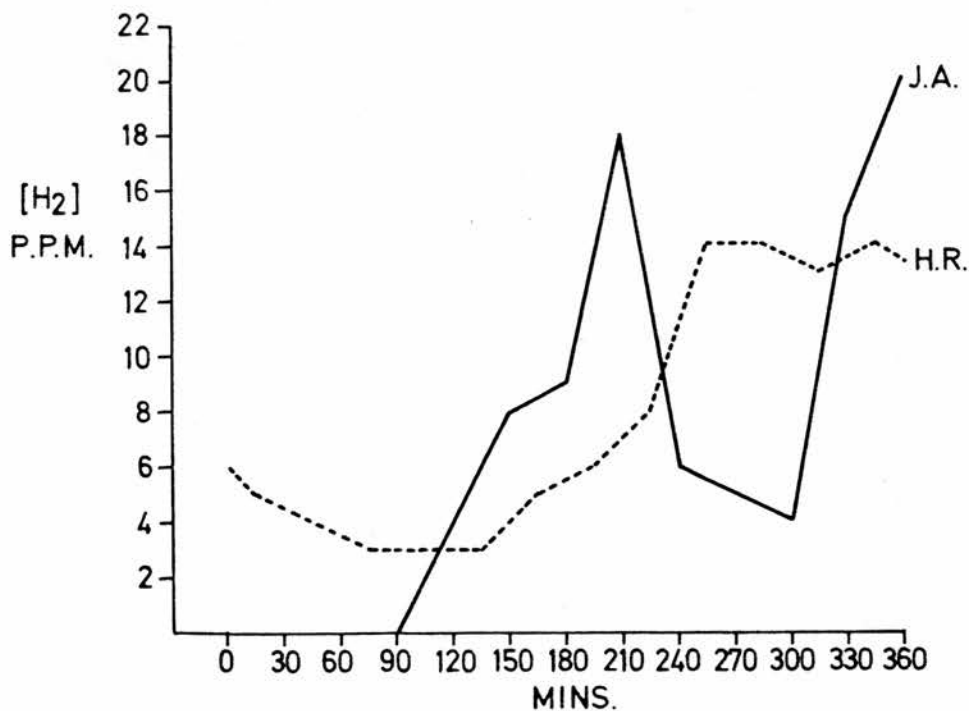


Fig.9: Breath hydrogen production in two male subjects previously exposed to G.A. for 3 weeks after administration of G.A. (25g) without glucose flavouring.

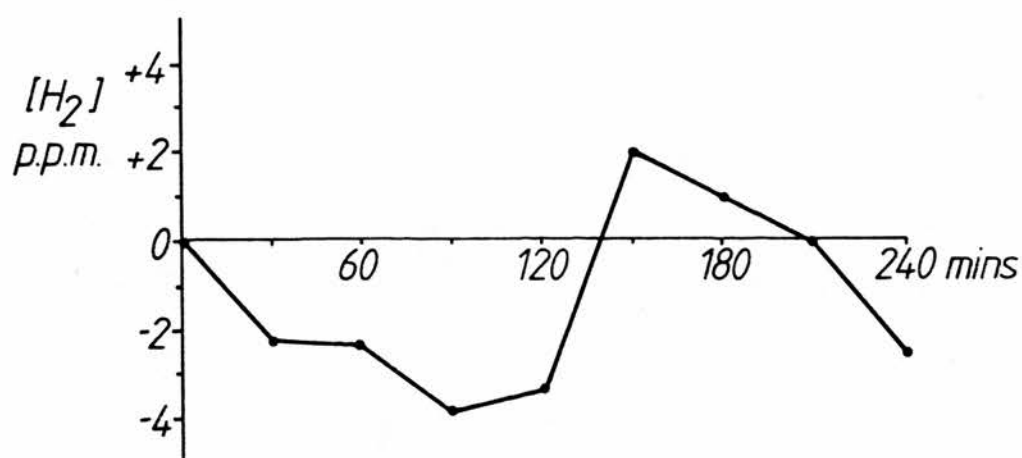


Fig.10: Breath hydrogen concentrations in male subjects (n=5) never previously exposed to G.A., given G.A. 25g (mean alteration in breath H₂ from concentration at 0 mins).

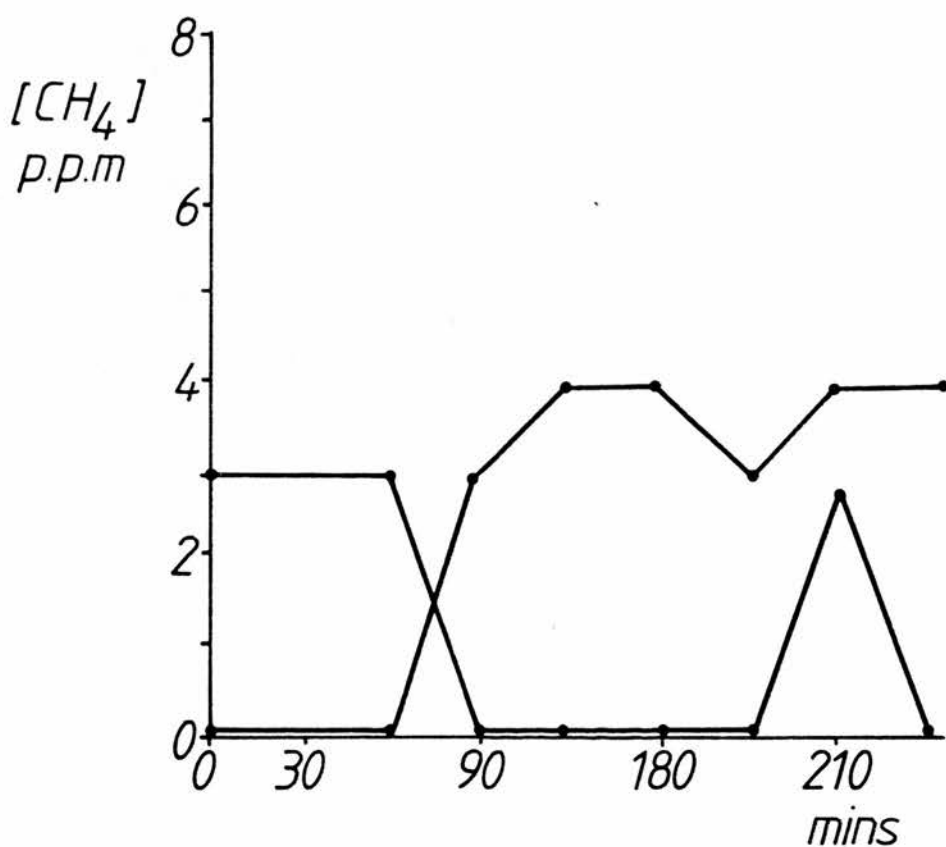


Fig.11: Breath methane response in a male subject before and after ingestion of G.A. (25g.d⁻¹) for 3 weeks.

Though many controversies persist, dietary fibre undoubtedly holds a place in the aetiology and management of many diseases of Western Civilisation. As such, a knowledge of the mechanisms of action and differing properties of varieties of fibre is of relevance to medical practitioners among whom surgeons are no exception. Indeed a number of conditions most commonly treated by the general surgeon have been linked, with varying degrees of certainty, to dietary imbalance and in particular dietary fibre deficiency. Thus diverticular disease, appendicitis and haemorrhoids, so called pressure diseases (Burkitt 1975), have been shown to have relationships with dietary fibre intake largely through epidemiological studies which compared diet and disease in Third World and Western countries. Thus in rural Africans who consume large quantities of dietary fibre the incidence of "pressure diseases" remains very low in comparison to more "civilised" populations where such conditions are common (Burkitt 1973, Burkitt 1971i, Burkitt et al 1974, Trowel 1977). The list of pressure diseases, many of which fill surgical clinics in the West, has been expanded to include varicose veins, functional bowel disease, and hiatus hernia though the link with dietary fibre deficiency becomes tenuous. Carcinoma of the colorectum is also a condition with epidemiological connections with fibre deficiency, being up to ten fold more common in Western Society than in rural Africa (Burkitt 1971ii), and becoming more common in societies where diet changes to Western tastes. Death rates from carcinoma of the colorectum show negative correlations with dietary fibre intake, a fact best illustrated in a comparison of urban Danes with rural Finns (I.A.R.C. 1977). Here urbanisation was related to considerably lower (44%) dietary fibre intake and a much higher incidence (c.400%) of colorectal carcinoma.

The suggested role for dietary fibre in the aetiology of disease has been expanded by enthusiasts to include the so called "metabolic diseases". Thus the prevalence of diabetes mellitus,

ischaemic heart disease, obesity, and cholelithiasis, have all been negatively correlated with the intake of dietary fibre.

Gallstones are more commonly found in women in Westernised society than in Third World countries. Thus in Scotland while nearly one third of elderly women will have gallstones at autopsy (Bateson and Bouchier 1975), a figure mirrored by results from other European centres, rural Africans have a far lower prevalence (c. 0-3%) (Owor 1964). As urbanisation proceeds and diet moves toward a "fibre deficient, refined carbohydrate excessive" form, diabetes mellitus is seen to become more common (Cleave 1974). Trowel (1976) has pointed to decreases in deaths from diabetes mellitus in Britain at times of dietary stringency when refined foods were unavailable and fibre intake consequently evinced as further support for the protective role of dietary fibre. Mortality rates for ischaemic heart disease can be shown to correlate with the incidence of carcinoma of the colon in nearly 28 countries (Rose 1974). Once again differences in dietary fibre intake may be shown to correlate with changes in the prevalence of ischaemic heart disease. Thus Indians of similar occupation and lifestyle had higher mortality rates from ischaemic heart disease when in communities which consumed low quantities of fibre (Malhotra 1971). Trowel (in Burkitt and Trowel 1975) has concluded that communities with low serum cholesterol levels and a low incidence of myocardial ischaemia tend to consume diets rich in dietary fibre. Furthermore Trowel has added to epidemiological studies, historical reviews (Trowel 1976). These indicate that in the U.K. fibre intake has in general fallen since the mid-19th century with a transient increase during food rationing in 1942-1953. Improvements in mortality from diabetes, ischaemic heart disease, and diverticular disease during the latter period of enforced fibre consumption were evinced as a further confirmation of the benefits of a fibre rich diet.

Such epidemiological and historical evidence has led certain workers to suggest that re-establishment of dietary fibre

supplementation compares with the expected effects on the incidence of pulmonary carcinoma if cigarette smoking ceases (Burkitt 1973). Such overenthusiastic support for the place of dietary fibre in the aetiology of disease ignores the considerable problems in interpretation of epidemiological and historical data. Errors in interpretation occur where many factors may be acting towards the aetiology of a disease. Thus pulmonary carcinoma, once included amongst diseases rare in high fibre consuming societies has more recently been properly linked with smoking status (Trowel 1977). Though uncommon in high fibre consuming societies carcinoma of the colorectum has been shown to have correlations with dietary fat intake (Wynder 1975), cholesterol intake (Liu et al 1979), meat consumption (Haenzel et al 1973) and in the case of carcinoma of the rectum to smoking (Doll and Peto 1976). Clearly ischaemic heart disease though possibly having links with dietary fibre depletion is more profoundly influenced by other dietary factors such as unrefined carbohydrate and fat consumption as well as by smoking (Doll and Peto 1976) and the stressful nature of Western life.

Nor may depletion of fibre from the diet be an isolated dietary event. Third World populations commonly suffer from malnutrition and parasitic diseases and these may play a major role in the subsequent development of disease (Eastwood et al 1976). Life expectancy, significantly reduced in Third World populations, may also influence the incidence of diseases of middle and old age such as diverticular disease and carcinoma of the rectum. Furthermore assumptions as to the dietary intake and bowel habits of studied populations may be erroneous. For instance the high stool weight associated with Africans and normally attributed to a high intake of dietary fibre may also be influenced by intestinal infestation or by ingestion of purgatives - a common practice among Africans (Eastwood et al 1976).

Finally the accuracy of diagnosis of diseases has improved and therefore makes for difficulties in establishing historical trends in the incidence. Thus diverticular disease

was little recognised by surgeons until the turn of the century (Telling and Gruner 1917), and appendicitis only recognised as such by the end of the 19th century (Fitz 1886). Inaccuracies in historical reporting may therefore lead to underestimates of the incidence of disease.

It seems then that while fibre undoubtedly is involved to a variable degree in the aetiology of disease caution must be exercised in ascribing cause and effect to one factor. The drawbacks of epidemiological or historical reviews are clear. From previous discussion the variable nature and effects of sources of dietary fibre are also evident. Such variety has led to calls for the study of well defined sources of fibre in isolation rather than in complex mixtures (Cummings 1981). In part this study has examined the feasibility and effects of the administration of a single complex polysaccharide in an otherwise fibre free diet. Furthermore it has attempted to examine suggestions that the long term effects of dietary fibre can, in practical terms, only be examined prospectively by using a satisfactory animal model (Hegstedt 1977).

The choice of gum acacia (arabic) as the "model" polysaccharide was governed by a number of factors+ 1. It is a polysaccharide in wide dietary use; 2. It has a well characterised and constant chemical structure and is readily identifiable using simple chemical methods; 3. As a dietary constituent regulatory bodies have sought clarification of its metabolic fate and effects, if any, on the host; 4. It is accepted as a water soluble dietary non starch polysaccharide generally agreed to be a substance included in the general definition of dietary fibre.

In view of the lack of information on the effects of chronic and subchronic administration of G.A. a 2 year multidose and a 90 day high dose study were conducted. In the 90 day, high dose, study the design was such as to ensure that daily intake averaged approximately $14\text{g.kg}^{-1}.\text{24h}^{-1}$ and this was achieved in both male ($14\text{g.kg}^{-1}.\text{24h}^{-1}$) and female ($13\text{g.kg}^{-1}.\text{24h}^{-1}$) groups. This level of G.A. intake is well beneath the known LD50 for rats (16g.kg^{-1}) (Bailey and Morgareidge 1976).

Growth rates were reduced in male rats but not in females. Female rats grow more slowly than males and may thus be less susceptible to marginal dietary inadequacies (Coates et al 1969). Goettsch (1948) demonstrated a similar relative resistance of female rats to dietary protein inadequacy when compared to male rats. Males on O.B.D. + 20% G.A. were only 78% the weight of their controls at the end of 90 days and this figure is close to that (75%) achieved by Shue et al (1962) after feeding G.A. (16% w/w over a period of time not stated by the authors. In the shorter term multidose feeding experiments, the quantity of protein and energy consumed by rats on 10% and 20% O.B.D. + G.A. diets was significantly less than that of controls. Elsenhans et al (1981) have also found significant reductions in food consumption in rats fed diets to which G.A. (10%, 20% or 40%) had been added. Weight was reduced compared to controls for 10, 20, 40% groups though weight gain was equally and most severely depressed by diets containing 20 and 40% G.A. It is recognised that rats require a minimum of 20% protein of high biological value in the diet for optimal growth (Coates et al 1969). Anderson, Farquhar and McNab (unpublished observations) have analysed the amino acid and protein base of the O.B.D. + G.A. diet used in these studies and have discovered suboptimal provision of protein above a level of 8.5% G.A. incorporation. (Table 1 Chapter 2.). Thus the O.B.D. + 20% diet only provides 188g.kg^{-1} protein. Furthermore amino acid analysis of the O.B.D. + 20% G.A. diet demonstrates significant deficiencies in a number of amino acids notably lysine, tyrosine, isoleucine and valine (Fig.2 Chapter 2). It therefore seems likely that reductions in weight are as a consequence of nutritional

inadequacy rather than any specific effect of G.A. itself. Interestingly a high dose of G.A. (20% w/w) administered to female rats, has drastic effects on breeding performance which are reversed following removal of G.A. from the diet (Anderson et al 1982). Furthermore the male female differences in weight gain seen at 90 days is abolished at 2 years when both males and females are found to weigh 11-15% less than their controls.

The reduction in renal and liver size, both absolute and corrected for animal weight in males and the reduction in absolute liver weight in females is again likely to be of nutritional origin. In related but separate experiments, multiple levels of G.A. were fed subchronically to rats (Anderson et al 1982). A linear relationship between degree of replacement of the diet by G.A. and liver weight was found. Similar effects on liver weight have been recorded in rats fed gum guar at doses of 1-15% for 13 weeks (Graham et al 1981), and after feeding raw potato starch (Fleming and Vose 1979). Nevertheless Booth et al (1963) found no alteration in organ weights after feeding G.A. (15%) to rats for 62 days. After gum guar, rats had absolute liver weights which were significantly less than controls at all levels with a similar pattern in males and females. Renal weights were reduced in males as were relative kidney weights though this effect was less prominent in females, possibly a reflection of their relative resistance to dietary inadequacy in the short term. Gum guar has been shown to cause reductions in weight gain in both male and female rats. These more pronounced effects of gum guar are probably linked to its considerably lower LD50 (7.06g.kg^{-1}) (Graham et al 1981). Despite reductions in relative size no histological evidence of hepatic or renal damage was evident. Furthermore no biochemical signs of hepato renal toxicity were found nor was hepatic protein synthesis affected. Urinalysis similarly demonstrated no signs of renal damage. Haematological parameters remained unaltered..

In addition to the normality of hepatic and renal histology no other significant histological abnormalities were

found in control or treated animals though caecal size did increase markedly in all O.B.D. + G.A. rats as a result of the fermentation of G.A. Caecal enlargement is a common finding when large quantities of undigested bulking agents are administered to rats whether in the form of inert agents such as kaolin (Dowling et al 1967); degradable chemicals such as sorbitan monooleate (Ingram et al 1978); or as dietary fibre in the form of potato fibre (Fleming and Vose 1979; Demigne and Remesy 1982), pectin, or cellulose (Hove and King 1979; Yang et al 1969). It is now considered that caecal enlargement is "fully attributable to normal physiological adjustment" and should not be considered as a toxic effect (Zbinden 1979).

In comparison to the final weights achieved by rats after 2 years on G.A. at various doses weight gain during short term administration of G.A. to adult rats was significantly greater though only the O.B.D. + 5% G.A. group which consumed greater quantities of protein and energy than controls. Tsai et al (1976) found no significant alteration in weight gain when rats were fed 5.7% G.A. The O.B.D. + 20% G.A. group gained significantly less weight than controls, in association with a reduction in food consumption. Increased weight gain after incorporation of bulking agents into the diets of rats is recognised to occur by stimulating hyperphagia as a result of caloric depletion from the diet. Elsenhans et al (1981) have demonstrated that the hyperphagic response is associated with ingestion of bacteriologically unavailable fibre such as carageenan, gum karya and tragacanth and absent when nutritionally available polysaccharides are consumed, possibly due to the caloric contribution of absorbed V.F.A. Thus cellulose added to a protein limited diet resulted in significant and linear increases in weight gain with graded increases in incorporation (Hove and King 1979). This effect was diminished when protein intake was limiting. Schneeman and Gallaher (1980) reported stable weight gain in rats fed 20% cellulose, as a result of hyperphagia. Pectin incorporation has been negatively correlated with weight gain (Hove and King 1979). Hyperphagic responses to massive levels of incorporation of inert diluents such as kaolin

have also been demonstrated, weight gain being maintained in adult rats, up to 66% incorporation but falling at higher levels (Dowling et al 1967).

Administration of G.A. to rats was carried out over a period of 2 years to ascertain whether any ill effects of prolonged polysaccharide intake might occur over such a period. It appears that no serious side effects occur, even at high levels of consumption. Premature death occurred equally in control and treated animals and the incidence of normal autopsy was very similar in both groups. Pathological abnormalities were little more common in treated rats (15%) than in control (10%).

The reduction in carcass weights once again reflects the inadequacy of nutrition in the high dose diets referred to in the discussion of the adult short term and 90 day immature feeding studies. On O.B.D. + 20% G.A. male and female final weights were similarly reduced compared to controls (11% and 12.6% respectively). It seems that the ability of immature females to compensate for nutritional deficiencies during the 90 day study was not maintained in the long term. Males however appear to adapt to the diet since percentage weight reduction compared to controls at 2 years (11%) is considerably less than that seen at 90 days (22%) (though of course dose related to animal weight would progressively fall with growth while G.A. incorporation in the diet remained static). Ross (1961) has demonstrated in a long term experiment that dietary inadequacies in rats which retard early development may be sufficient to allow normal long term development and that such restriction may even prolong life.

Though nutritional deficiencies resulted in lower body weights no signs of this were reflected in haematological or biochemical abnormality. In particular serum protein and albumin levels were unaffected by increasing doses of G.A. indicating that while somatic protein depletion may have occurred, visceral protein synthesis and breakdown remain balanced. As in the subacute study liver and renal function were unimpaired. Neither male nor female

rats demonstrated altered serum calcium levels and bladder calculi were present in only two animals. Furthermore no nephrocalcinosis was present histologically after 90 days on high dose G.A. Short term experiments in man have indicated the potential for some sources of dietary fibre to induce negative calcium balance. Thus bran may induce negative calcium balance (Cummings et al 1979; Pebson et al 1975; Heaton and Pomare 1974, Rheingold et al 1976). It has been suggested in addition that iron absorption may be impaired by wholewheat products and bran (Jenkins et al 1975) though results in rats fed white, brown and wholemeal bread of constant phytate content suggest this is solely a phytate mediated effect (Fairweather-Tait 1982). Faecal sodium and potassium increases after a high vegetable and fruit diet or after bran (Stasse-Wolthius et al 1980) and similar alterations occur in magnesium, zinc, and phosphorus excretion (Rheingold et al 1976). However if mineral balance is examined this is frequently undisturbed since increased faecal excretion parallels increased mineral intake in the fibre consumed (Van Dokkum et al 1982). Vitamin absorption in relation to fibre consumption has been little studied but high doses of cellulose and pectin have been shown to reduce vitamin B₁₂ absorption by rats (Cullen and Oace 1971). Folate absorption is probably unaffected by cellulose, pectin and bran from studies of in vitro binding and absorption using a chick bioassay (Ristow et al 1982). Clearly these effects have been demonstrated largely for particulate, water insoluble and relatively undigested fibre and may be related to their properties of cationic binding (McConnel et al 1974), water holding, or gel forming (Eastwood and Kay 1979). Pectin, a highly water soluble, bacterially degraded polysaccharide causes no alterations in sodium, potassium, magnesium, or calcium excretion in man (Stasse-Wolthius et al 1980) despite its strong cationic affinity. All these studies are relevant to the acute administration of fibre but of greater importance is the effect of individual fibres on long term balance (Cummings 1978). This study demonstrates that G.A. has no long term clinical, biochemical haematological effect on these.

The long term effects of G.A. administration to rats in terms of effects on intestinal morphology and cellular kinetics has been studied since it is suggested that alterations in these could result in some of the effects on small intestinal function caused by fibre. In the animals studied only one demonstrated a gross morphological abnormality in the form of subtotal villus atrophy the aetiology of which remains unclear. The isolated nature of this lesion makes it unlikely that it was an effect mediated by gum arabic. No dose related effects on villus height were found to indicate a tendency toward atrophy with G.A. nor was crypt size affected. However crypt cell production rate was significantly reduced at the highest dose level of G.A. and showed a linear reduction with increasing dose of G.A. Since in normal circumstances a dynamic equilibrium exists between crypt cell production and cell loss from the villus (Williamson 1978) this suggests that G.A. results in reduced villus cell exfoliation to compensate for the reduction in crypt cell production. Many factors can alter small intestinal morphology and these include the pattern of feeding, the quality or quantity of food, and the rate of its ingestion (McManus and Isselbacher 1970). Increasing age reduces cell turnover, and diurnal alterations also occur. Small intestinal resection is the most potent cause of increased mucosal cell turnover (Williamson 1978).

Alterations in morphology and kinetics are governed by feedback controls whereby cell production in the crypts is governed by the complement of cells in the villus, such that stimulation to production will occur when villus cell numbers fall below a critical number (Rijke et al 1974). Williamson (1978) has summarised the numerous, interdependant, factors which may exert control over the small intestinal mucosal morphology and kinetics. Intraluminal nutrition and the presence of combined pancreaticobiliary secretions exert trophic effects on the jejunal and mid ileal mucosa. Local or topical effects on the mucosa are accompanied by humoral influences on intestinal mucosa manifest in results of cross circulation experiments. Many potentially enterotrophic hormones including gastrin, enteroglucagon, pituitary

hormones acting through adrenal, thyroid and gonadal target organs exist. Some hormones such as V.I.P. exert antitrophic effects. Though bacterial action is unlikely to exert a trophic effect, altered blood supply possibly mediated through catecholamines may also be of importance.

Dietary inadequacy and in particular fasting has been shown to have a marked effect on small intestinal mucosal morphology, kinetics and function (McManus and Isselbacher 1970). After a short (15-16h) fast small intestinal weight falls as a result of loss of mucosal bulk. The absorptive capacity of mucosa increases but this is accompanied by no change in villus morphology. However after more prolonged fasting (up to 10 days) villus height and depth of mucosal crypts fall (Clarke 1970 ii; Clarke 1972; Altman 1972) the reduction in villus height being most prominent in the proximal small intestine with little effect on the distal mucosa (Clarke 1972) where villus height is less, possibly as a result of the reduced trophic effect of intraluminal nutrients consequent upon their absorption proximally. Crypt cell production rate falls by 20% in fasting rats (Aldewachi et al 1975; Clarke 1970ii), the maximal reduction taking approximately three days to be reached (Altman 1972). This reduction in crypt cell proliferation is achieved by a slowing of the cell cycle (Aldewachi et al 1975) and is reversed by feeding (Altman 1972). Villus height is restored by refeeding but crypt depth, though it may increase transiently (Aldewachi et al 1975) remains depressed for up to seven days after commencement of refeeding (Altman 1972). In this study O.B.D. + 20% G.A. resulted in reduction in protein and energy intake with lowered final carcass weights at two years. It is therefore possible that the alterations seen in small intestinal cell production were simply secondary to dietary nutritional inadequacy. However the constancy of the villus height and crypt depth argue against undernutrition as the sole aetiological factor. It must also be recognised that the nutritional status of rats, starved for up to seven days, in which weight loss was of the order of 20% and in which alterations in mucosal morphology and kinetics were observed (Clarke 1970ii)

were greatly different from the rats in this study. Indeed the short term experiments on weight gain in adult animals indicate an increase in weight of the O.B.D. + 5% fed group.

Though protein calorie deprivation has been studied closely the effect of dietary fibre on mucosal morphology has been less well documented. A fibre free elemental diet administered to rats has been reported to cause decreased crypt size and crypt cell production (Nelson et al 1978; Lehnert 1976; Ecknauer et al 1981). Villus size has been reported to increase, probably due to decreased shedding of enterocytes from the villus tip (Lehnert 1976; Nelson et al 1978) though others have reported opposite findings (Ecknauer et al 1981). Jacobs (1981) studied the effects of three fibre sources, pectin, oat fibre and gum guar on villus morphology and kinetics. Pectin (10% w/w) reduced villus height, decreased crypt cell production, and increased crypt depth, findings similar to those after feeding 18% pectin though at this higher dose villus height, though increased significantly at one point in the jejunum, remained essentially unaltered (Brown et al 1979). Guar and oat bran caused no significant alterations in villus height or crypt depth when added to a fibre free diet though oat bran may have reduced crypt cell production (Jacobs 1981). No studies of the effects of gum arabic have previously been published.

No differences in mucosal villus morphology were seen during this study between controls and rats on G.A. supplements. Owen and Brandborg (1977) have reported alterations in the mucosal patterns of habitual vegetarians towards the broad branched fused villi of normal height seen in subjects from developing countries. Evidence suggests that certain sources of fibre may induce the maturation of intestinal villi at weaning (pectin) while others (cellulose) do not possess this property (Tasman-Jones et al 1982). Electron microscopic evidence goes further to suggest that mucosal denudation of the villus tips of rats may occur after consumption of fibre. Cassidy et al (1981) have found increasing degrees of mucosal damage at the villus tip after the consumption of cellulose,

bran, pectin and alfalfa. The increasing extent of mucosal damage was correlated with the bile binding capacity of the fibre sources examined. The suggestion that bile binding activity might be responsible for mucosal damage are supported by studies of bile binding resins of which cholestyramine, the most powerful, produced maximal intestinal mucosal damage (Cassidy et al 1980). Such mucosal damage has been suggested as a possible factor in the effects of dietary fibre on small intestinal function. Alterations in villus height and cell turnover may also significantly affect absorptive function and may be part of the explanation for the delayed effects on glucose absorption evident after weeks or months on pectin, cellulose, or bran (Schwartz and Levine 1980, Brodribb and Humphreys 1976).

From these studies G.A. has a minor but significant effect on small intestinal mucosal kinetics but no effect on morphology, and in these respects is similar to published results for oat bran (Jacobs 1981) which contains high concentrations of water soluble polysaccharides. If as has been proposed mucosal alterations form part of the mechanism whereby fibre exerts its effects on small bowel function, then these minimal changes accord with the lack of systemic effects on mineral and vitamin balance seen in the rat and man in the short and long term.

Metabolic effects of the short term administration of gum arabic in man.

Gum arabic administration resulted in minimal effects on the metabolic activities of human subjects. In particular cholesterol and glucose homeostasis were studied since previous work has demonstrated that dietary fibre may alter them.

All subjects in this study demonstrated a reduction in serum cholesterol at the end of the study. Such a hypocholesterolaemic effect has been noted for a number of fibres, notably those that are water soluble, such as pectin, guar, bengal gram and oat

fibre (De Groot et al 1963, Durrington et al 1976; Jenkins et al 1975; Kay and Truswell 1977; Mathur et al 1968; Kirby et al 1981). The method by which fibre may produce its hypocholesterolaemic effect appears to vary. Malabsorption of ingested cholesterol may occur resulting in a rise in faecal neutral sterol excretion. Such an increase is seen after pectin ingestion which produces a marked hypocholesterolaemic response (Kay and Truswell 1977; Stasse-Wolthins et al 1980). Bile acid excretion may also increase in association with hypocholesterolaemia after pectin and oat bran ingestion (Kay and Truswell 1977; Cummings et al 1979iii; Kirby et al 1981). This may result in reductions in serum cholesterol concentrations in two ways. Firstly, by reducing the bile acid pool consumption of cholesterol may increase to replenish lost bile acids. Secondly reduced bile acid presence in the intestinal lumen may impair the ability to absorb cholesterol. However alterations in bile acid homeostasis are not inevitably translated into effects on cholesterol metabolism. Hence bran, recognised in general to be relatively ineffective in producing reductions in serum cholesterol (Truswell and Kay 1976; Eastwood et al 1973) has on a number of occasions been demonstrated to increase bile acid excretion (Cummings et al 1976; Findlay et al 1974; Cummings et al 1979ii). Similarly bagasse has no effect on total cholesterol and yet results in an increased excretion of bile acids and an insignificant reduction in neutral sterol output (Walters et al 1975). Similar discrepancies have been noted in the rat (Vahouny et al 1980). The ability of some fibre sources such as gum guar to reduce post prandial glucose and insulin responses (Jenkins et al 1978) has been linked to their demonstrated ability to reduce serum cholesterol (Jenkins et al 1975; Kahn et al 1981). High levels of serum glucose and insulin may drive the liver to produce cholesterol and their reduction, by the presence of fibre, may result in hypocholesterolic effects (Kahn et al 1981) (Jenkins et al 1978).

G.A. had no effect on either faecal bile acid or neutral sterol excretion in this study, nor did any significant alteration in glucose or insulin homeostasis occur after its administration.

There are therefore no indications as to the mechanisms whereby cholesterol levels are lowered by G.A. ingestion by humans.

Studies of cholesterol homeostasis in the rat were not carried out since there is a belief that it is a poor model for investigation (Hegstedt 1977). Nevertheless some information on gum arabic and lipid metabolism is available in animals though none has previously been available in man. Tsai et al (1976) studied the effects of pectin, carrageenan, agar, cellulose, wheat bran and gum arabic in rats. While pectin, as in man, proved to have a major hypocholesterolaemic effect reducing serum, aortic and liver tissue levels gum arabic (5-7% in the diet) resulted in inconstant results with an increase in serum levels after 56 days. Increases in liver cholesterol were seen and whole body cholesterol was thought to be increased also. These results contrasted with these of Kiriyaama et al (1969) who noted a moderate hypocholesterolaemic effect for gum arabic (5%) after only 5 days on a hypercholesterolaemic diet. Tsai et al explained the discrepant results on "dietary interactions" noting that small increases in fat intake abolished the hypocholesterolaemic effect of pectin. Two other studies have suggested effects of G.A. on cholesterol absorption. Labelled cholesterol absorption from the rat intestine was noted to be reduced by gum arabic (5%) though less so than by pectin (Kelley and Tsai 1978). G.A. produced no significant lowering of serum cholesterol in contrast to pectin but did increase tissue levels, and cholesterol biosynthesis by a minor amount, and failed to alter cholesterol turnover. This reduction in cholesterol absorption confirmed similar though less marked findings in the rat fed small doses ($0.5\text{g}\cdot\text{d}^{-1}$) G.A. with cholesterol supplements ($50\text{mg}\cdot\text{d}^{-1}$) (Lin et al 1957). Animal studies therefore suggest that while G.A. may alter cholesterol absorption and metabolism, it appears to have inconstant effects on serum levels in the rat. A further possible factor is the knowledge that G.A. increases the rate of small intestinal transit though absorption of ^{14}C marker is unaffected (Frape et al 1982). Phospholipid and triglyceride levels were unaffected by G.A. ingestion. In comparison to

to cholesterol, studies on these two elements of serum lipids are few and in general little effect on serum levels has been observed. Though Heaton and Pomare (1974) have reported reductions in serum triglycerides after bran ingestion, other studies have demonstrated the inefficacy of guar (Kahn et al 1981), bagasse (Walters et al 1975), wheat bran (Jenkins et al 1975; Eastwood 1969; Walters et al 1975) and oat bran (Kirby et al 1981). Lipoproteins have been little studied though pectin and cellulose have been reported to lower serum levels in adults (Durrington et al 1976), and children (Shurpelaker et al 1971).

The inability of G.A. to influence lipoprotein and triglyceride levels is mirrored by its lack of effect on post prandial rises of glucose and insulin. Though mean glucose and insulin concentrations were rather higher after G.A. ingestion the differences were at no point significant. Other gums, and in particular gums tragacanth and guar are highly effective at reducing post prandial insulin and glucose responses, and are more so than insoluble fibre such as methyl cellulose and wheat bran (Jenkins et al 1978). However gum arabic solution is of low viscosity and therefore different from tragacanth and guar whose high viscosity correlates closely with their hypoglycaemic effects in man (Jenkins et al 1978) and rats (Blackburn and Johnstone 1982) and with their ability to limit glucose diffusion. They used a modified glucose tolerance test but it is unlikely that the simple G.T.T. used here will have significantly altered glucose responses to G.A. supplementation. Glucose malabsorption is not believed to produce the hypoglycaemic effect though xylose absorption was slightly impaired after guar and tragacanth (Jenkins et al 1978). It is not possible to say whether any glucose malabsorption occurred in this study in view of the breath hydrogen response to G.A. but clearly if it occurred it was in quantities insufficient to alter blood glucose levels. One further factor of relevance is that G.A. and the oral glucose load were taken one after the other. It is known that the effect of fibre is reduced if it is simply scattered over food rather than mixed intimately with it

(Williams et al 1980). However since G.A. is highly water soluble intimate mixing is likely to have occurred. The same authors have pointed to the great differences between the modified or standard glucose tolerance test and the ingestion of a regular meal. Nevertheless G.A. appears to have had no effect on glucose tolerance under similar conditions to those under which other dietary fibres produce a degree of glucose reduction. The administration of G.A. for three weeks prior to the G.T.T. is of some importance since evidence exists that chronic administration of cellulose and pectin in rats, and bran by humans may produce a delayed effect on glucose tolerance (Schwartz and Levine 1980; Brodribb and Humphries 1976).

Apart from a uniform reduction in total cholesterol no other biochemical abnormalities were noted in subjects fed G.A. for three weeks. Mean serum ALT and AST concentrations fell during the three weeks on G.A. to levels that remain within the limits of a normal distribution for AST though slightly below normal for ALT in four of the five volunteers. Other parameters reflecting aspects of liver function were undisturbed. Total protein and albumin were minimally and insignificantly increased (mean increases $+ 2.8 \pm 2.78$; $+ 1.0 \pm 1.73 \text{ g.l}^{-1}$ respectively) reflecting satisfactory hepatic synthesis. Bilirubin and alkaline phosphatase concentrations were similarly minimally altered. Serum calcium was not altered significantly.

Renal function remained normal as judged by serum creatinine, urea and total CO₂ which remained static before and after G.A. ingestion. Similarly electrolyte balance was undisturbed judged by serum levels. No adverse effects of G.A. upon haematological parameters were seen though in three weeks it is unlikely that any alterations subsequent upon dietary imbalances would have been noted.

G.A. was well tolerated by all subjects and though all noted the passage of increased quantities of flatus only three felt occasionally distended and in none was this a distressing feature. Feelings of intestinal cramp and bloating are common after dietary fibre ingestion and may limit its therapeutic usefulness (Anon 1981).

Gum arabic had little effect on faecal characteristics. Mean mouth to anus transit time was unaffected by G.A. ingestion. Increases were seen in four subjects and a decrease in the fifth. Two of the four whose M.A.T.T. was increased had control transit times of less than 36 hours and it has previously been demonstrated that fibre may lengthen M.A.T.T. where initially it is rapid (Brodrick and Humphreys 1976; Harvey et al 1973). The failure to influence transit time is not surprising since this is a property largely attributable to particulate insoluble fibre such as bran which is reduced by cooking or by refinement (Heller et al 1980; Wyman et al 1976). Water soluble fibre however has little effect on faecal dynamics and thus pectin has been shown to have no effect on intestinal transit (Kay and Truswell 1977; Durrington et al 1976; Cummings et al 1979iii). Similar findings have been noted in rats (Vahouny et al 1980).

Similarly faecal weight was unaltered by the consumption of G.A. Here again particulate fibre such as bran or vegetable fibre result in increases either through their water holding capacity (Eastwood 1973) or as a result of bacterial proliferation (Stephen and Cummings 1981). Once again the effects of gel forming or water soluble polysaccharides are less marked than those of

particulate dietary fibre. Guar gum (20g.d^{-1}) produced a 20% increase in faecal weight while the same dose of apple carrot cabbage or bran increased wet weight by 40%, 59%, 69% and 127% respectively. Similarly pectin has been shown to increase wet weight by small but significant amounts in two studies (Cummings et al 1979iii; Durrington et al 1976) and dry weight in one (Kay and Truswell 1977). Since pectin is completely degraded during passage through the large bowel (Cummings et al 1979iii) increases in faecal weight may have been caused by increased faecal water, bacterial proliferation, or by increased quantities of volatile fatty acids. In this study a significant correlation was found between V.F.A. excretion and faecal wet weight. V.F.A. has previously been suggested as a mediator of stool weight (Williams and Olmstedt 1936ii; Hellendoorn 1978) but it is generally felt that the association between V.F.A. and stool weight is coincidental (Cummings 1981ii). Clearly if extensive fermentation of G.A. has occurred in the caecum as a result of bacterial activity little evidence of this is seen in alterations in stool weight and yet cabbage which is extensively degraded during large bowel transit (Cummings et al 1978ii) results in marked increases in stool weight 35% of which is represented by bacteria (Stephen and Cummings 1980i). In the rat experiments, significant increases were found in both wet and dry weight after O.B.D. + 10% G.A. consumption. Increased food intake on this diet may have contributed significantly to these increases. G.A. was not detected in the faeces however and the evidence from the faecal weights on elemental diet demonstrates that G.A. is almost completely degraded during large intestinal passage ruling out the presence of intact G.A. as a cause of the increases. Estimations of the water contents of O.B.D. and O.B.D. + G.A. faeces were considered to be too prone to error through dehydration prior to faecal collections. Bacterial proliferation may well have contributed to the increase in faecal weight since in this instance large quantities of G.A. were consumed by rats ($6.7\text{g.kg}^{-1}24\text{h}^{-1}$) in comparison to the relative consumption by human subjects ($c.0.4\text{g.kg}^{-1}24\text{h}^{-1}$). The elemental diet dry weight increased by 42% in the absence of

any fibre in the diet and on the assumption that no G.A. was present in the faeces and in the knowledge of the great increase in caecal weights observed it is reasonable to attribute this weight increase to bacterial proliferation. Administration of G.A. to rats has previously been described as producing a bulky stool (Booth et al 1963) (Lin et al 1957). The latter authors attributed this to the appearance of undigested G.A. in the stool, though no proof of this was offered.

The metabolism of gum arabic in rat and man

From these studies it appears that G.A. is extensively broken down during passage through the gastro intestinal tract as a result of bacterial fermentation.

In the rat recovery experiments from various points in the gastro intestinal tract indicate that G.A. remains substantially intact during passage through the stomach and small bowel since precipitants obtained from the small bowel retained a typical migration pattern of G.A. monomers after acid hydrolysis. This would accord with attempts at G.A. digestion by human alimentary secretions in vitro. However the technique clearly cannot exclude minor degradation of the molecule during upper intestinal transit. Some evidence from human experiments in ileostomists suggests that a minor degree of degradation of fibre may occur in the small bowel (Sandberg et al 1981; Holloway et al 1978; Holloway et al 1983). Furthermore though a typical G.A. precipitate did not appear from large bowel contents the method used cannot exclude the presence of an extensively degraded and water insoluble residual molecule in the faecal content. Hydrolysis and chromatography of the meagre gelatinous precipitate obtained from the caecal and colonic contents was quite unsatisfactory. Definition of the multiplicity of sugars was poor and gave no indication of alterations in the presence of gum arabic monomers, and it is therefore impossible to rule out the presence of a partially degraded gum arabic molecule in the water soluble phase. Vercellotti et al have confirmed such difficulties with standard chromatographic

techniques. The similarity in quantity of the precipitants obtained from control and gum treated large bowel content suspensions suggest that if present such degraded molecules are in small quantity. These limitations aside, the faecal weight measurements in the elemental diet experiments support evidence for the extensive degradation of G.A. Average daily intake of G.A. on this diet could only be estimated. Weight gain on the diet was very close to that on O.B.D. pellet diet however and an allowance of 25% for food loss by scattering is probably generous. Nevertheless if this figure is accepted, G.A. intake was $c.3g.d^{-1}$. Daily faecal weight was greatly reduced whilst on Elem-D or Elem-D + G.A. in agreement with many other studies on elemental diet in humans (Winitz et al 1970ii) and in the rat (Grenstein et al 1957). Despite a daily intake of 3g G.A. only a minimal increase in daily dry weight of faeces occurred ($0.3g.d^{-1}$). Elsenhans et al (1981) have reported similar increases following G.A. supplementation of an elemental diet. On the basis of this increase G.A. degradation appears to be in the order of 90% in the rat on an elemental diet. Increases in faecal weight were seen on O.B.D. + 10% G.A. for both dry and wet weight. However both food consumption and fibre intake were significantly increased in the O.B.D. + 10% G.A. group during this experiment and these are very likely to have contributed to the increase in faecal weight. Other possibilities are that water content increased or that bacterial proliferation as a result of G.A. fermentation occurred (Stephen and Cummings 1979, 1980ii).

Relative caecal weight, increased significantly when G.A. was added to either the elemental or pellet diet. Such increases are commonly seen after addition of bulking agents and have been described after pectin and cellulose administration to rats, the increases being linearly related to polysaccharide dose and highly significant (Hove and King 1979).

Volatile fatty acid production was studied to assess the site and extent of fermentation. The distribution of V.F.A.

concentrations was similar to that found in previous studies in rats (Remesy and Demigne 1976; Elsdon et al 1946). Gastric concentrations were not estimated in view of the known tendency of rats towards coprophagia. Small intestinal concentrations were negligible indicating minimal fermentive activity. Caecal concentrations were maximal with stepwise reduction in concentrations between the caecum, distal colon and faeces.

The maximal and significantly elevated concentration and content of V.F.A. in the caeca of rats fed O.B.D. + 10% G.A. and the subsequent reductions in colonic content and faeces indicate that this organ represented the major site for G.A. fermentation. Similar increases in V.F.A. concentrations have been recorded in the rat after pectin and cellulose ingestion (Hove and King 1979; Yang et al 1969) with significantly higher levels after pectin which is known to be completely degraded during intestinal transit in man (Cummings et al 1979iii); Demigne and Remesy (1982) have demonstrated that increases in the V.F.A. pool after potato fibre (25% w/w) were secondary to increases in caecal size with little alteration in concentration. The importance of the caecum for the digestion of G.A. is emphasised by the considerable reduction (59%) in faecal concentrations of V.F.A. after caecectomy in rats subsequently fed O.B.D. + 20% G.A. Precipitable G.A. appeared in the faeces having been undetectable in the stool of anatomically intact animals on O.B.D. + 20% G.A. Yang et al (1969) demonstrated a 13% reduction in cellulose digestibility after caecectomy in the rat but provided no information on V.F.A. production. Remesy and Demigne (1976) reported a more pronounced reduction in V.F.A. concentrations in the faeces of rats fed a standard diet. This may relate to the lower availability of fermentable polysaccharide or possibly to the time after caecectomy which would influence the ability of the colon to metabolise the fibre. Rats in this study were not investigated on a control diet. The appearance of precipitable G.A. in the faeces indicates a marked reduction in digestibility. It had been hoped that studies on an elemental diet might allow quantification of the reduction in digestion but rats were unable to tolerate the diet. Though digestion of G.A. is

reduced V.F.A. concentrations in the faeces of caeectomised rats on O.B.D. + 20% G.A. were comparable to those of intact animals on O.B.D. Clearly caeectomy does not abolish V.F.A. production in the rat. It is known that compensatory colonic growth occurs after caeectomy (Scarpello et al 1978) and this is likely to increase the capacity for colonic fermentation. Other facts may contribute to the reduction in V.F.A. concentration and output. A tendency towards looseness of the stool was common after caeectomy and could have reflected a reduced intestinal transit time which may cause a reduction in polysaccharide fermentation, a factor suggested by other authors (Southgate and Durnin 1970; Cummings et al 1973). Undigested gum arabic may in itself promote increased transit time and by a bulking action reduce the faecal V.F.A. concentration. Alternatively, since the caecum and right side of colon represent a primary site of water absorption which is impaired after caecal excision in the rat (Scarpello et al 1978) increased stool water may have diluted the V.F.A. content.

An elemental diet to which G.A. could be added was developed to produce a nutritionally acceptable diet to which could be added fibre sources whose metabolic fate could then be studied in isolation. Despite a fibre free diet caecal V.F.A. concentrations were similar to those found in O.B.D. fed rats and only slightly higher than those recorded by Thomson et al (1982) on elemental diet (allowing for 75% water content of caecal contents). Dietary differences and times of sampling in relation to feeding are probably sufficient to explain these differences since marked diurnal variations in V.F.A. concentrations are known to occur in rats (Remesy and Demigne 1976). Though concentrations are similar to those found in O.B.D. fed animals the V.F.A. content of the caeca is greatly reduced when rats are fed Elem-D or Elem-D + G.A. to 41.7% and 59% of values on O.B.D. respectively. In the absence of unabsorbed dietary fibre the likely substrates for production of V.F.A. are mucopolysaccharides and glycoprotein hexosamines from small intestinal mucus and desquamated mucosal cells (Vercellotti et al 1978).

Mucus hexosamines are found in considerably elevated concentrations (x30-50) in the dilated caeca of germ free rats (Lindstedt 1965). Furthermore bacteria are present in faeces which will ferment these compounds (Salyers et al 1978, Salyers 1979) and bacterial contamination of the intestine of the germ free rat abolishes caecal distension (Gustaafsson et al 1970). In addition unabsorbed oligosaccharides are likely to provide a contribution. It is known that in intubated humans 2-4% of an oral sucrose load will pass into the caecum where it is fermented (Bond et al 1980). In contrast to the findings on O.B.D. and O.B.D. + G.A., caecal V.F.A. concentrations were reduced in the Elem-D + G.A. group of rats (these figures represent the combined results of two separate studies of V.F.A. concentration under identical conditions). A marked increase in caecal weight on Elem-D + G.A. resulted in a significantly higher V.F.A. content in these animals however. This rather unexpected finding does not relate to a simple dilutional effect since water content was higher in the Elem-D caecal contents (87% vs. 76.9%). Thomson et al (1982) have noted similar findings in rats on elemental diet to which pectin was added.

Faecal V.F.A. concentrations and daily output showed a similar pattern for both pellet and elemental diet with increases in both with G.A. supplementation. As with caecal content V.F.A. output was considerably less in the elemental diet situation. V.F.A. output and faecal V.F.A. concentration rose with increasing doses of G.A. in the pellet diet and concentrations rose in a linear fashion. The rise in V.F.A. output was statistically insignificant as it was for Elem-D + G.A. These findings contrast with previous results in man where rises in faecal V.F.A. concentrations have only been described after pectin (Spiller et al 1980), cabbage and cellulose ingestion (Ehle et al 1982). Daily V.F.A. output in contrast increases after a wide variety of dietary fibre ingestion by human subjects (Rubenstein et al 1969, Cummings et al 1976, Spillar et al 1980, Williams and Olmstedt 1936ii, Hellendoorn 1978). The relative

proportions of V.F.A. altered during intestinal transit, after G.A. ingestion, and with changes in the nature of the diet. The most striking change in V.F.A. proportions was seen after Elem-D and Elem-D + G.A. administration. Elem-D resulted in the appearance of isobutyrate, isovalerate and valerate in the caecal content and faeces whereas these were absent in Elem-D + G.A. animals. Isomeric forms were absent in intestinal content and faeces of O.B.D./O.B.D. + G.A. fed animals and valerate was only occasionally detected. The findings confirm those of Thomson et al (1982) who detected almost identical V.F.A. patterns with disappearance of isomeric forms and valerate on adding another complex polysaccharide pectin to an elemental diet. Other studies in rats do not report the presence of these minor V.F.A. when animals consume normal or fibre supplemented diets (Remesy and Demigne 1976; Yang et al 1969; Hove and King 1979). Certain bacteria are known to be dependent upon such isomeric forms, e.g. *Ruminococcus bromii* (Bryant 1974) and their appearance implies their altered production or consumption or modification of the bacterial flora. The lowered caecal V.F.A. concentrations of rats fed elemental diet may be linked therefore to lowered production rates in the face of an altered caecal environment. Rubenstein et al (1969) fed an effectively elemental diet to human subjects for four days. While dialysable V.F.A. concentrations dropped no mention of altered patterns of V.F.A. was made. Increases in acetate proportion and reduction in butyrate were seen during intestinal transit and occurred on both elemental and pellet diets. This accords with previous findings in the rat (Remesy and Demigne 1976; Yang et al 1969) though in the former study propionate proportions were in excess of butyrate, the situation when elemental diet was administered in this study. Yang et al (1969) noted a more pronounced reduction in butyrate concentration in the faeces (15-6%) with a proportionate increase in acetate (73%-88%) than seen in this study. The alterations in V.F.A. proportions might be due to differential absorption. Dawson et al (1964) on the basis of V.F.A. infusion into a single patient with a mucous fistula concluded that absorption rate was dependent upon V.F.A. chain

length. However further, and more satisfactory, studies using a dialysis bag technique (McNeil et al 1978) or intubated volunteers (Ruppin et al 1980) suggest that absorption rates are similar for the three major V.F.A. and that absorption occurs at similar rates in the proximal, mid, and distal colon (McNeil and Cummings 1979). Alternatively increased production of acetate in the distal colon and rectum or the increased utilisation of butyrate either by bacteria or the colon itself could result in altered V.F.A. proportions. In vitro evidence exists that butyrate is a preferred energy source for isolated colonocytes (Roediger 1980) though injection of acetate, propionate and butyrate into the rat caecum appears to result in the rapid disappearance of each at equal rates (Buchanan et al 1943).

Increased incorporation of G.A. in O.B.D. resulted in a linear decrease of butyrate proportions and a similar increase in acetate proportion. In contrast Yang et al (1969) demonstrated no alteration of either Acetate/Propionate or Acetate/Butyrate ratio in faecal V.F.A. after cellulose administration. Elemental diet not only results in the appearance of minor V.F.A.s but also produces a reversal of the butyrate:propionate ratio seen in O.B.D. fed animals. Elem-D + G.A. administration results in reductions of both acetate/propionate and acetate/butyrate ratios in contrast to the effect of O.B.D. + 10% G.A. and to the increases seen after the addition of pectin to an elemental diet (Thomson et al 1982). Similar differences between the caecal V.F.A. profiles exist after Elem-D and O.B.D. diets. O.B.D. + 10% G.A. increases acetate/propionate and acetate/butyrate ratios but the reverse happens with Elem-D + G.A. This situation for O.B.D. + 10% G.A. is similar to that seen in the caecum after cellulose administration to rats (Yang et al 1969) and pigs (Argenzio and Southworth 1975), and in the equine large intestine where acetate and propionate are related in a reciprocal fashion (Argenzio et al 1974). These findings confirm that dietary interactions have profound effects on the metabolic fate of dietary polysaccharides in the rat and as in the ruminant are major factors in the alteration of V.F.A. metabolism (Stevens 1978). This and other studies indicate that patterns

of fermentation in the large bowel are influenced not only by the nature and quantity of polysaccharide consumed but in addition by the constituents of the diet they are contained in and by the species consuming them. Further evidence of the effects of diet on caecal metabolism was seen in measurements of exhaled hydrogen and methane. Significant decreases in hydrogen and increases in methane production were in contrast to the findings in man after G.A. consumption, indicating altered pathways of metabolism. The passage of large quantities of unabsorbed carbohydrate into the caecum is known to favour lactate production (Torres-Pinedo et al 1966) and it is possible that the much greater consumption of G.A. by rats than man may predispose to anaerobic conditions more favourable to methane production. Elemental diet abolished hydrogen and methane production which, with reduction in V.F.A. production, reflects the reduction in available fermentable substrate. The delay seen in reappearance of these exhaled gases after addition of G.A. is presumably accounted for by an adaptation period required by the producing bacteria to re-establish themselves in the new caecal environment. Conflicting evidence exists as to the effects on bacterial populations of the human colon during elemental diet feeding. Though Winitz et al (1970ii) has described marked reduction in concentration of faecal bacteria other workers have not confirmed this (Crowther et al 1973) (Burnside and Cohn 1975). However reductions in strictly anaerobic species have been noted after elemental diet feeding for seven days (Attebery et al 1972) in contrast to increases in these species after fibre supplementation (Fuchs et al 1976) and may go some way to explaining altered intestinal gas production on elemental and regular diets. Further evidence of bacterial adaptation is seen in the progressive rise in methane production over four weeks in the rat.

The relative importance of the rat caecum is again emphasised by the abolition of methane and hydrogen excretion after its excision, this contrasts the importance of the caecum in man which is claimed to produce less than 10% of total methane production (Levitt and Inglefinger 1968). Clearly hydrogen and methane production are more dependent upon the caecum in rats than

V.F.A. production which continued after caecectomy and though one caecectomised rat excreted hydrogen this was associated with a sub acute anastomotic obstruction which results in increased breath hydrogen excretion in man (Levitt 1969). The production of methane after G.A. ingestion and the importance for its production suggest that in these respects the caecum may more closely resemble the rumen than the human colon.

Metabolism of G.A. in man.

G.A. appears to be substantially untouched by human alimentary secretions, and prolonged incubation with gastric and pancreatic juices results in no reduction in the precipitable weight of G.A. Variability in precipitant weight in excess of anticipated weight has been taken as evidence of mucopolysaccharide or mucoprotein precipitation from the digestive solutions which occurred in control solutions. Chromatographic analysis of the supernatants might in the event of gum arabic degeneration have been anticipated to reveal the presence of constituent carbohydrate monomers. These were not detected and though oligosaccharides cleaved from the G.A. molecule may have been present it appears that G.A. remains substantially intact when incubated with the alimentary secretions of man, though minor damage to side chains cannot be excluded from these experiments which could alter the subsequent behaviour or handling of the polysaccharide (Cummings 1981i).

In vivo, the administration of G.A. resulted in evidence of extensive degradation. Faecal weight did not increase significantly after the consumption of G.A. (25g) to the diet although this is a very inaccurate assessment of digestibility. Wyman et al (1978), Slavin and Marlett (1971) have demonstrated the tremendous variability of faecal excretion in apparently healthy subjects. Attempts to precipitate G.A. from faecal slurry produced identical precipitates from control and test period faeces. Recovery experiments have demonstrated that G.A. could be precipitated from the aqueous phase of a faecal slurry at the

anticipated G.A. concentrations were the molecule to pass through the alimentary tract untouched. Clearly this method is insensitive to small amounts of G.A., and to the presence of insoluble remnants of a partially degraded G.A. molecule. However the responses in breath hydrogen excretion confirm that metabolism of G.A. is occurring. These increases only occurred in subjects who had taken G.A. for a period of three weeks and were absent in individuals who had no previous exposure to large doses of G.A. The possibility that the rises of hydrogen excretion were due to the passage of malabsorbed glucose passing into the caecum has been excluded by the finding of increased breath hydrogen after G.A. ingestion alone by two subjects. Concentrations were not as high as those recorded after lactulose, raffinose or stachyose (Tadesse 1980), the infusion of lactose into the terminal ileum (Levitt 1969) or the administration of beans (which contain high concentrations of raffinose and stachyose) (Steggerda 1968). Nevertheless increases were greater than those seen after the ingestion of carrot fibre (6g.d^{-1}) for three weeks although single measurements only were made in that study (Robertson et al 1979). Breath hydrogen production occurred rather later than previously reported following lactulose administration. Taking mouth to caecum transit time as that point at which breath hydrogen increased 10 p.p.m. above baseline values then this was not reached until 210 mins. compared to means of 93.9 to 73.6 minutes after 10-20g lactulose recorded by La Brooy et al (1983). The mean time for first rise of breath hydrogen was 120 minutes. This is longer than that recorded by Tadesse et al (1980) for lactulose (66 ± 2 mins.) similar to that for raffinose (115 ± 33) and shorter than after stachyose (185 ± 18 min.). It is therefore possible that G.A. results in a slowing of mouth to caecum transit time though an alternative possibility remains that the delay is caused by time taken for the degradation of the complex molecule into fermentable units. This latter suggestion is supported by the knowledge that Jenkins et al (1978) found gum guar to increase mouth to caecum transit time (+ 75 mins.) but that hydrolysed, non-viscous guar, had no effect. The lack of breath hydrogen response in subjects previously unexposed to G.A. is of interest and raises the possibility of induction of bacterial

fermentive activity. Similar responses to acute ingestion of complex carbohydrate have been noted previously. Increased breath hydrogen excretion after carrot fibre consumption were not seen until after ten days on the diet (Robertson et al 1981) and cellulose, lignin and pectin similarly produced no effect on breath hydrogen excretion in acute feeding experiments (Tadesse and Eastwood 1978). Marthinsen and Fleming (1982) have reported that 2-3 days are required before stable levels of breath hydrogen could be recorded on xylan, pectin, cellulose, corn bran and fibre free diets and suggested that this represented a period of bacterial adaptation to the diet. In vitro studies do not support this adaptive argument. Fermentation of fibre by faecal suspensions taken from subjects accustomed to a high bran intake showed no greater hydrogen production than faeces from subjects whose intake of fibre was low (Bond and Levitt 1978).

An alternative explanation might lie in a delayed response in hydrogen production. Staggerda (1968) demonstrated in vitro that hydrogen production only commenced at 6-8 hours for oligosaccharides but within 2-3 hours for mono and disaccharides. However Tadesse et al (1980) have demonstrated that the infusion of raffinose and stachyose into a colostomy resulted in rapid production of hydrogen in the colon though no increase in breath hydrogen occurred. Delays in breath hydrogen response after oral administration of these oligosaccharides were attributed to altered small intestinal transit though delay in their caecal metabolism was not excluded. Evidence that hydrogen production was not increased up to 10-12 hours after acute cellulose, pectin, and lignin ingestion suggests that a late rise in production after G.A. was not missed in this experiment (Tadesse and Eastwood 1978).

The digestibility of fibre appears not to affect hydrogen production in the limited studies available. Pectin produced no hydrogen response after acute administration (Tadesse and Eastwood 1978) and although Marthinsen and Fleming (1982) have demonstrated

higher levels of breath hydrogen after chronic ingestion these levels were insignificantly different from those produced on a fibre free diet.

Delays in hydrogen response after consumption of complex polysaccharides may therefore result from an initially reduced ability to degrade the molecule to its component oligosaccharides rather than an inability to utilise these for hydrogen production.

Methane production was unaltered by acute administration and abolished or reduced by chronic administration of G.A. This finding is consistent with previous experience showing methane excretion to be unaffected by ingestion of a variety of carbohydrates. (Levitt and Inglefinger 1968, Tadesse and Eastwood 1978). Marthinsen and Fleming (1982) have found reductions in breath methane production after bran and though increases were seen after xylan consumption concentrations were no higher than those on a fibre free diet. McKay et al (1981) found a correlation between non cellulosic pentose consumption and breath methane concentrations. Though arabinose, a component of G.A., resulted in increased breath methane excretion, administration of pentose rich polysaccharides in the form of oranges, bran, carrot and apple caused no alterations in methanogenesis.

Metabolism of G.A. caused no alterations in faecal V.F.A. concentrations or excretion. In man faecal V.F.A. concentrations seem relatively little affected by fibre ingestion though pectin, cabbage and cellulose have been recorded to induce increases (Spillar et al 1980; Ehle et al 1982). V.F.A. output, however, has been demonstrated to increase by pectin (Spillar et al 1980), bran (Williams and Olmstedt 1936i; Cummings et al 1976), cellulose (Williams and Olmstedt 1936i; Spillar et al 1980), beans (Hellendoorn 1978) and a variety of vegetables (Williams and Olmstedt 1936ii). Of note was a delay in the elevation of faecal V.F.A. concentrations after cabbage ingestion. Concentrations insignificantly altered at two weeks rose significantly by six weeks raising again the possibility of adaptation

and induction of fermentive bacteria.

The correlation between stool weight and V.F.A. is interesting since V.F.A. has been implicated in the determining of stool weight (Williams and Olmstedt 1936i; Hellendoorn 1978). Fernandez et al (1971) found a strong correlation between V.F.A. and stool weight in patients with diarrhoea but it is likely that V.F.A. is related to faecal weight only in a casual manner (Cummings 1981). In this study V.F.A. output was correlated with intestinal transit time. Previous suggestions that increases in the rate of intestinal transit may reduce the digestibility of fibre have been made on the basis of studies in patients with post resectional diarrhoea (Cummings et al 1973) and in elderly subjects presumed to have slower transit times (Southgate and Durnin 1970). These may not be relevant in the context of this study. Though G.A. and pectin appear to be highly degraded during intestinal passage they result in differing effects on faecal V.F.A. despite G.A. being consumed in greater quantity. However, measurements of V.F.A. in the faeces is a blunt indicator of fermentive activity in the large bowel. Ready absorption of the principal V.F.A. occurs from the colon (Dawson et al 1964; Ruppin et al 1980) and rectum (McNeil et al 1978; McNeil and Cummings 1979) and it is estimated that only 1.2-10% of V.F.A. produced is excreted by man (calculated from figures in Cummings 1981).

From these studies therefore it appears that administration of G.A. to the rat results in nearly 100% degradation as a result of bacterial action manifest by alterations in the production of methane and volatile fatty acids. These activities proceed predominantly in the caecum. In man recovery experiments, despite their imprecision, supported by alterations in hydrogen production suggest extensive metabolism of G.A. during intestinal transit. Little or none of this degradation is likely to occur before passage of G.A. into the caecum.

Summary.

1. G.A., a complex polysaccharide, highly resistant to in vitro chemical hydrolysis and regularly consumed in variable quantities by man, is extensively degraded during intestinal transit in the rat and man. These findings are in accordance with similar findings for other water soluble, non-particulate sources of dietary fibre such as pectin and gum guar.

2. The degradation of G.A. in the intact rat is confined chiefly to the caecum though after its removal fermentation of complex polysaccharides continues to a lesser degree in an adapted colon. Determination of the relative importance of various sites in the human large bowel has obviously not been possible but evidence from the literature indicates that the caecum is of significantly less importance for fermentation in man. In both the rat model and in man degradation of G.A. in the small bowel appears to be absent or minimal.

3. Fermentation of G.A. is marked by alterations in breath gas excretion by both man and the rat. However while hydrogen excretion is increased in man and methane excretion reduced the opposite pertains in the rat. The importance of the caecum in the rat is emphasised by the abolition of gas production after caecectomy. Differences in breath gas responses in the rat and man may be caused by species differences in microbial populations, altered metabolic pathways consequent upon the differing quantities of G.A. administered in the rat and human experiments, and the predominance of the caecum in rat fermentation. Volatile fatty acid production is stimulated by addition of G.A. to the diet and although faecal excretion is rendered difficult to interpret because of intestinal absorption, at high doses it appears to relate to ingested polysaccharide dose in the rat. Altered V.F.A. profile caused by G.A. appears to be a highly variable finding between fibre sources, though the absence of complex polysaccharides in the caecal content alters pathways of metabolism and results in the appearance of

V.F.A. otherwise absent in the rat. Though absent in the rat on a regular diet these minor V.F.A. are a normal part of the human V.F.A. spectrum.

4. There is a suggestion that fermentive activity for G.A. must be induced before alterations in breath gas excretion occur. This finds some support from previous studies of breath gas and V.F.A. responses after fibre consumption. Degradation of the complex molecule may also result in delay in appearance of the hydrogen response compared to its appearance after oligosaccharide administration.

5. G.A. has little metabolic effect on man. Its hypocholesterolaemic effect is similar to that of other sources of D.F. particularly those soluble in water or gel forming. These studies do not elucidate a mechanism for this effect. Its lack of effect on glucose/insulin homeostasis is compatible with its low viscosity. There are no long or short term ill effects from the ingestion of G.A. in various doses by the rat.

6. In contrast to water insoluble, particulate, and relatively undigested fibre and in agreement with properties of other water soluble polysaccharides G.A. has no significant effects on colonic function. It is possible that small bowel transit is prolonged by G.A. but the evidence suggests that this may be an effect due to delayed degradation of the G.A. molecule.

7. G.A. has no effect on small intestinal mucosal morphology and a minimal effect on mucosal cell turnover and this might be in keeping with its inert nature in terms of small bowel function.

8. The behaviour of gum arabic is therefore compatible with previous evidence of the properties of other water soluble or gel forming polysaccharides, though it appears to have minimal effects on the metabolic activities of the host in the long and short term.

9. It appears that G.A. is equally greatly degraded in humans and in the rat. However there can be little doubt of the major role of the caecum in the rat and these studies support previous suggestions of its relative importance compared to man. Whether this influences the ultimate fate of gum arabic is however less clear. The difference in response in gas production point to differences in metabolic or bacterial environment in the rat and human large intestine and further evidence of these differences is found in the subtle alterations of V.F.A. profile seen between the two species. These differences do not seem sufficient to contraindicate the use of the rat in the study of the metabolism of fibre isolates or their mixtures.

10. Elemental diet administration alters caecal metabolism measured by breath gas excretion and the profile of V.F.A. The addition of a polysaccharide into the caecum appears to restore the metabolic status quo. The elemental diet used here has maintained growth rate and reproduced preliminary findings from other studies. Added polysaccharide is well tolerated and appears to be handled similarly to when it is added to a standard diet. As might be predicted from studies in germ free animals V.F.A. production continues when elemental diet is administered, probably as a result of the presence of endogenous polysaccharide in the caecum. This diet therefore appears to be an attractive method of studying the metabolism of D.F. and the effects of interactions between its various sources.

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